

Economics, Animals and Pollution

PROFESSOR HARRY JOHNSON, economist, American, holder of chairs at the London School of Economics and the University of Chicago, and a former member of the now defunct Council for Scientific Policy, has come down hard on the so-called environmentalists. In a well written and instructive booklet prepared for the benefit of the British-North American Committee, Professor Johnson brings the power of economic reasoning and, it might be said, common sense to the problems of man and his environment, and his arguments deserve to be read by all who profess to have at least a passing interest in these problems (*Man and His Environment*, British-North American Committee, £0.40).

It seems that there are still people who need convincing, or even teaching, that economic growth produces something other than pollution. Those who advocate bringing the economy to a standstill so that further damage to the environment can be avoided do so, it seems, without fully realizing the implications. Quite often it is these very same people who, quite properly, call for all efforts to be made to improve the lot of people living in the developing countries. But how can this be achieved without economic growth? The advantage of having professional economists turning their attention to these problems is that the most perceptive of their profession soon come up with the answer that growth and preservation of the environment are not necessarily incompatible.

The arguments for this are by now well publicized and Professor Johnson reiterates them to effect: "Economic growth and not the impediment of it is the way to a betterment of the quality of life and the avoidance of ecological disaster." He argues, quite cogently, that what are often considered the chief environmental problems of today—air and water pollution, deforestation, apparent shortage of mineral resources—are not the most important ones. Air pollution can be decreased by attacking its source, either by technical developments which are already available, or by continuing research into, for example, making the internal combustion engine more efficient and therefore less of an environmental hazard. Water pollution can similarly be tackled while forests can be replanted and minerals recycled. This is not to say that these programmes of work will present no problems, but there is no evidence to show that the ingenuity of man will not, as it has so often done in the past, provide a solution.

There are, however, other problems to which the environmentalists have attached their flag. And it is these which are more deserving of attention. Wild animals and fish once they are hunted to extinction are a permanent loss and no amount of technology (or genetic engineering for that matter) will bring back extinct species. It is in this field that there is need for the greatest concern. But in some cases, clearly, a compromise will have to be reached where the animal provides a living for some people or groups of people and where a wholesale ban of hunting would not be totally beneficial.

A case in point is the concern now being expressed once again for the survival of whales worldwide. There are those who would ban whaling for several years to ensure

that some species which are most threatened will increase their stock without danger. But is there a need to jeopardize the livelihood of so many fishermen and others who make their living out of whale products in order to preserve these aquatic mammals?

Would not a more sensible approach be to try and extend the ban already in existence on some whale species to others which are in danger? To ensure the success of such an approach all the whaling nations, the largest of which are Japan and the Soviet Union, must cooperate to the full, and even though such an approach might be difficult to police, it will stand a much better chance of being approved by the International Whaling Commission which meets in London at the end of the month than a blanket ban on whaling.

A far more worthwhile campaign than attempting to have a moratorium on whaling would be to persuade the whaling nations which are not members of the commission to either become members or observe the quotas and bans imposed by the commission.

Professor Johnson, quite properly, is more concerned with the conservation of animals than pollution, at least in so far as he sees solutions to the latter problems, but his faith in science which is evident in most of his booklet deserts him when it comes to problems which cannot transparently be subjected to economic analysis. "It is not established," he says, "that we have the scientific capacity to remedy the damage we may be doing to our oceans and upper atmosphere by treating both as a costless medium for the transport of people and goods and the oceans as an inexhaustible source of fish and crustaceans for human nourishment." Where, Professor Johnson, have you lost your faith in the ingenuity of man?

100 Years Ago



A PROJECT has been set on foot by Colonel Grant, so well known from his African travels, to form a loan exhibition of skulls and horns of hollow-horned animals, in order that by observation and comparison of a large number of characteristic specimens, facts may be obtained regarding the form, sexual characters, and locality of each particular species. It is proposed to have as many as from twenty to fifty specimens of each species, so as to be able to form groups representing every stage in the life of each, as also to show the varieties of species in different localities. When from three to five thousand specimens of the one hundred and fifty existing species has been promised, means will be taken to secure the most suitable place in London for their exhibition.

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OLD WORLD

Forging Links Between Steelmaking and Nuclear Power

THE linking together of steelmaking and nuclear power is now a sufficiently real prospect, albeit ten years or more away, for representatives of European steel and nuclear companies to have decided in principle to form a club to look into the possibilities and problems more fully. This move was made at a discussion meeting, convened last week in London by the British Steel Corporation and the British Nuclear Forum, at which it was announced that Japan is to spend about £10 million on research into nuclear steelmaking during the next six years.

Although the meeting left the details of the club—who should be eligible for membership, how much money should be spent and precisely what the objectives should be—to be decided in future consultations, it did provide a useful opportunity for airing the differences between what the steelmakers require of a nuclear power plant and what the nuclear people think they can offer.

One of the basic reasons for the steel industry regarding nuclear power as an attractive source of energy is that the coking-coal now used to produce the coke for blast furnaces is likely to become considerably more costly and scarce in the future. And of the longer term alternatives—such as replacing some of the coke in a blast furnace by oil or natural gas or converting other coals to coke—the most attractive seems to be direct reduction of ore by a hydrogen and carbon monoxide gas.

The so-called reforming stage, at which hydrocarbons are converted into suitable reducing gases, requires heat at 900° C or so and this is where a nuclear plant in the form of a High Temperature Reactor (HTR) would come in. Helium coolant at a temperature of about 1,000° C could be used as the transfer medium and reducing gases could be produced at a temperature of about 800° C. This temperature is critical because if the hydrogen is any colder the reduction of ore pellets in the favoured "continuous shaft process"

can take excessive times and reduction of the pellets may even then not be complete because of reoxidation. (On the other hand, at higher temperatures the ore pellets have to be carefully selected because of the problem of "sticking".)

What do those concerned with HTRs have to say about the problems? Dr L. Shepherd, head of the OECD Dragon HTR project, said that helium temperatures of 1,000° C can now be produced for long periods at Dragon with little difficulty and that it should ultimately be possible to raise this by perhaps a few hundred degrees. He went on to say that the design of heat exchangers capable of operating at the temperatures and pressures required seemed to be the most difficult problem. Ceramic exchangers—made, for example, of silicon carbide or silicon nitride—would be

lighter and cheaper than metal ones but their performance at high pressures may prove to be a stumbling block.

Another factor that has to be taken into account is that an integrated nuclear steelworks would have to be near a populated area and therefore safety would be a paramount consideration. Because of this, most of the discussions at the meeting assumed that there would be a secondary helium circuit to ensure that radioactive contamination would be minimal. Fortunately the HTR is considered by many to be the safest available reactor type. There is another possibility, however; reducing gases could be produced with reforming plant at a remote nuclear site and then pumped cold to a steelworks, where they could be reheated by conventional means or by self-burning.

SELECT COMMITTEE

Science Adviser Likely

THE Select Committee on Science and Technology may be about to acquire a full-time science adviser. Mr Airey Neave, chairman of the committee, is understood to have approached Mr James Prior, leader of the House of Commons, asking for his comments on various options put forward by members of the committee.

Although the committee has not discussed the proposal formally as yet, there has been pressure from a number of members for a full-time secretariat. The proposal—if it is accepted—would move the select committee a little nearer the Congressional committee system in the United States where the powerful House committees often have highly qualified secretariats which write and commission background reports for the committees' members.

If Mr Prior approves the scheme, the science and technology committee will be the first of the select committees to appoint a full-time adviser. Such an appointment would not, however, replace the *ad hoc* appointments that the committee makes from time to time—for instance it is advised on computer matters by Professor A. Douglas of the London School of Economics.

As the committee has yet to formally consider the proposal and as Mr Prior has not yet replied to Mr Neave, the precise role of the adviser has still to be defined. It is unlikely, however, that he

would be an academic scientist. Rather the select committee is likely to look for someone who can find out government attitudes on various matters for the committee.

In line with the select committee's desire to ensure that Mr Heath's government is in fact "open government" it is likely that the correspondence over the appointment of the adviser will be published, if in fact approval for the appointment is given.

Meanwhile the committee is finalizing its reports on nuclear reactor policy and computers. Both will be published in about a month's time, as will the committee's report on the hovertrain. When that appears it is more than likely that Mr Michael Heseltine or, conceivably, Mr Peter Walker, will be invited by the committee to comment on its findings. For a vehicle that no longer runs the hovertrain seems to be an issue with plenty of mileage left in it.

The committee also hopes to finalize its plans to visit Brussels shortly. The visit, originally planned for May, will now take place in the week beginning July 9. It is probable that the committee will take evidence from Commissioners Dahrendorf and Spinelli, as well as from Mr Christopher Layton, Director for Advanced Technology Industries at the EEC. It is also likely that the select committee will see the commission's directors on energy and nuclear power, and a day visit to Ispra, one of the laboratories of the Joint Research Centre, may be included in the itinerary.

Nature Subscriptions

THE *Nature* subscription list has been computerized. Each address label now carries a reference consisting of three letters and four numbers. Any queries relating to subscriptions should in future quote this reference.

ANIMAL BREEDING

Bullseye

THE first deep-frozen bull has been born. Late last week a healthy bull calf was born to a cross bred Hereford-Friesian cow at the Agricultural Research Council's Unit of Reproductive Physiology and Biochemistry at Cambridge. Nine months ago the embryo of the calf was immersed in liquid nitrogen having been removed, after fertilization, from the uterus of its true mother.

The bull calf is the first large mammal to be born after being deep-frozen as an embryo. If the technique is perfected it could have large commercial implications for the livestock industry.

In all, the Cambridge unit transplanted twenty-two eggs into eleven cows some nine months ago. The eggs all came from normal mothers fertilized by artificial insemination, and were removed from the uterus on day 10 of pregnancy, in the blastocyst stage.

The eggs were then frozen to -196°C with dimethylsulphoxide, a cryoprotective (antifreeze) agent, replacing some of the water in the embryo cells to prevent ice forming within them. Having been cooled very slowly at 0.2°C a minute they were kept frozen for six days. They were then thawed at a rate of 360°C a minute in a 37°C water bath, and transplanted into the uterus of the host mother.

Only two of the eggs became implanted in the host uteri. Six weeks into pregnancy one cow was developing two embryos, but one of these died later in the pregnancy. Nonetheless, one bull was born successfully.

This success with cattle follows the development of the freezing technique with mice in the summer of last year by the Cambridge unit and by the Oak Ridge National Laboratory in the United States. The mouse and cattle programmes were in fact running virtually in tandem, but the faster rate of reproduction in mice naturally brought success there before success with cattle, which have a gestation period of about nine months. Similar experiments with pigs and sheep have not as yet been successful.

The failure with ten of the cows is attributed to damage to the embryos during freezing and thawing.

Dr Wilmut, one of the research leaders at the Cambridge unit, said this week that the freezing and thawing process undoubtedly kills some of the embryo cells. Because some differentiation has occurred in the blastocyst stage it is probable that in the unsuccessful attempts a number of vital cells were killed, whereas in the successful attempt the cells killed were replaceable.

Experiments already carried out with

earlier stages of embryo development have not been successful, possibly because of the presence of the zona pellucida, a relatively tough membrane which surrounds the ovum and which complicates the freezing process.

Future experiments, Dr Wilmut said, will involve varying four factors—the stage of development at which the embryo is removed from its true mother, the rate at which the embryo is frozen, the rate at which it is thawed, and the composition of the medium in which it is frozen. Any one of these factors is crucial.

The potential value of the technique, however, makes careful experiments that may lead to a commercial system well worth while.

If perfected commercially, milk cows could be used to breed beef cattle. The saving would lie in not having to keep beef cows alive purely for breeding purposes. Milk cows could still be used for milk production before and after they carry beef cattle.

Equally the technique would allow genetic characteristics that are in danger of being bred out of cattle for economic reasons to be kept in cold storage for the day when these characteristics may again become commercially important. At present if certain gene strains are

bred out then there is no way they can be recovered.

Entire herds could be transported around the world in liquid nitrogen flasks, and countries such as Australia, which are still free of certain European diseases, could import frozen embryos that time would have shown to be disease free, whereas at present cattle imports are strictly limited.

The technique would also allow for a careful record of genetic change to be kept, as frozen embryos could be developed decades after the eggs were first fertilized. The resulting animal could be compared to the types that have developed by natural selection. This could in time improve selection methods in cattle breeding.

But all this may be many years away. Developing a commercial freezing method with a high success rate could be a long job, and various refinements to the peripheries of the technique will be needed before frozen embryos become commercially available. For example a non-surgical method of transplanting the embryos will have to be devised as surgery is time consuming and expensive. But these problems are likely to prove quite minor. The problem that really needs solving is how to guarantee a high success rate.

SOVIET SCIENCE

Of Machines and Men

from our Soviet Correspondent

LUNOKHOD-2, which was rolled out on to the lunar surface on January 16, 1973, has completed its working programme after a life of five lunar "days" in which it traversed some 37 kilometres (approximately 3.5 times as far as the less mobile and less manoeuvrable Lunokhod-1).

Although in many respects the Lunokhod-2 programme continued its predecessor's work—spectrometer and penetrometer investigations of the chemical and physical properties of the surface rocks, the investigation of solar and galactic corpuscular radiation, and a continuation of the Franco-Soviet laser range-finding research to determine the exact distance from the Earth to the Moon (see *Nature*, **228**, 795; 1970)—certain features of the programme appear to be new.

The choice of landing site, on the eastern edge of Mare Serenitatis, enabled a detailed television study to be made of the transition region between mare and highland. In all, over 80,000 television pictures were received, with a stereoscopic system providing detailed interpretation of the relief—a matter of especial importance during the investigation of a major tectonic fault on the fourth working "day" (April 11–22, 1973).

Special emphasis, too, was given to the magnetic experiments. The greater range of mobility of Lunokhod-2 enabled variations in the magnetization of the surface rocks and variations of the lunar magnetic field to be measured over a fairly extensive track.

Another innovation was the use of laser direction-finding apparatus to determine the luminosity of the lunar sky in the visible and ultraviolet regions.

According to Academician Boris N. Petrov (*Pravda*, March 25, 1973), the Lunokhod programme of automatic lunar exploration, with its potentialities for long-term and long-range study of the surface, is envisaged as one of the main projects of the Soviet Space Programme, equal in importance with the Saylut series of orbiting space stations. Academician Petrov is closely connected with the planning of both types of research (he is one of the chief organizers of the proposed Soyuz/Apollo flight, and is chairman of the IFAC Committee on Automatic Control in Space) and may therefore be assumed to speak with detailed knowledge of both branches. In view of the recent setback to the Saylut programme, however (see *Nature*, **243**, 181; 1973), and the undoubted success of Lunokhod-2, a rethinking of priorities in Soviet space planning may well be in progress, with even greater emphasis being given to automata.

NEW WORLD

Change of Tack on the Shuttle

by our Washington Correspondent

THE long and convoluted debate about the space shuttle has taken a strange and confusing turn. A Congressional agency has taken a damaging swipe at NASA's economic justification for the project, but NASA officials, undaunted, have come up with a fresh analysis claiming that the shuttle is an even better economic bet than they had previously thought. In Congress, meanwhile, critics of the shuttle are arming themselves with a new set of arguments to use when NASA's budget comes up for debate in the Senate, but even the project's staunchest opponents are privately conceding that they have little chance of choking off the funds this year, at least.

What it all adds up to is more confusion in an already confused situation. By now, the shuttle is perhaps the most intensely studied technological project to come before Congress, but as an example of prudent science policy making, the whole debate leaves a lot to be desired, chiefly because it has been concerned almost exclusively with the economics of the project while its wider implications have been underplayed. The real issue is the shape and balance of the US space programme for the rest of the century.

Congressional opponents of the shuttle, led by Senator William Proxmire and Senator Walter Mondale, last year set the General Accounting Office the task of examining the basis of NASA's claims that development and use of the shuttle will save the agency money when compared with the cost of launching satellites with conventional, expendable rockets. The GAO has some harsh things to say about NASA's figures, but its report, published last week, is remarkable chiefly for the fact that it reveals for the first time that NASA has recently come up with a completely new economic analysis of shuttle operations.

When the final shuttle design was chosen last year, NASA argued that although it would cost some \$8,100 million to develop and produce, use of the shuttle would save some \$5,200 million between 1978 and 1990. The analysis rested on the assumption that NASA's budget would be able to support 581 shuttle flights in that period, and that the chief savings would result from the fact that the shuttle will enable satellites to be repaired and refurbished after they had been placed in orbit, and from removal

of size and weight constraints in payload development. But, on April 27—three days before the GAO originally intended to publish its report—NASA sent the accounting office a completely new mission model. Instead of 581 shuttle flights up to 1990, the agency is now suggesting that its budget will be able to support 779, and the estimated savings have shot up from \$5,200 million to about \$16,000 million.

GAO has not had time to examine these drastically modified estimates in detail, and the effect has been to torpedo, to some extent, its analysis of NASA's original estimates. The new mission model has neither been explained in detail by NASA nor subjected to independent analysis, but according to Mr Dale Myers, head of the Office of

Manned Spaceflight, one of the chief differences from the original model is that it assumes that one third of the shuttle flights will be sortie missions, using the spacelab which will probably be developed by the European Space Research Organization (see *Nature*, **241**, 418; 1973).

The spacelab is a pressurized capsule in the shuttle payload bay, which will house up to six scientists, and an unpressurized unit that will be used for large instruments such as telescopes and antennae. The unit will remain attached to the shuttle throughout the mission and will be removed on landing and prepared for the next flight. Mr Myers said in a telephone interview last week that the spacelab is expected to reduce payload costs substantially, so that more

AIR POLLUTION

Relief for Detroit

by our Washington Correspondent

THE Environmental Protection Agency last week published some data to back up its assertion that nitrogen dioxide does not present a widespread air pollution problem in the United States. In fact, the agency now reckons that only two cities have levels of nitrogen dioxide that pose a potential hazard to health, while a year ago 47 regions were accorded that distinction. Sighs of relief were clearly audible in Detroit at the EPA's reappraisal of the situation.

The reason for the rather dramatic change in the status of nitrogen dioxide is that the method that was originally used to measure concentrations of the pollutant in the air has recently been found to have given greatly elevated readings. The result is that only Los Angeles and Chicago are now reckoned to have levels of nitrogen dioxide greater than $100 \mu\text{g}^{-3}$, the level above which it is considered to be harmful to health. There is also some question about New York and Salt Lake City, where the EPA is rechecking its measurements.

The importance of all this for the automobile manufacturers is that the Clean Air Act commands them to produce cars in 1976 which emit 90 per cent less oxides of nitrogen per mile than those produced in 1971. That requirement was based on the original faulty measurements, and the manufacturers are now claiming that such stringent controls are not necessary. In order to meet the requirements of the act, the

car makers would have to fit a second catalyst to the conventional engine, in addition to the converter needed to remove hydrocarbons, and a committee of the National Academy of Sciences reported recently that there is considerable doubt about the reliability of the dual catalyst system.

The EPA's new analysis suggests that even if no stricter automobile emission controls than those now in operation are applied, only Baltimore, in addition to Los Angeles and Chicago, will have levels of nitrogen dioxide in 1985 that exceed the $100 \mu\text{g}^{-3}$ level. The EPA is allowing 45 days for comments on its new analysis, after which it will turn all the data over to Congress with a suggestion that the requirement for 90 per cent reduction in exhaust emissions be modified. The chances are that Congress will probably end up modifying the Clean Air Act so that car makers will still have to reduce emissions of oxides of nitrogen substantially, but not to the extent of fitting dual catalyst systems to car exhausts.

In the meantime, however, the EPA must hold public hearings and decide whether or not Chrysler should have an extra year to meet the present standards prescribed by the act. As with hydrocarbon emissions standards (which are not affected by the reappraisal of oxides of nitrogen) the EPA Administrator can grant manufacturers an extra year to comply if he finds that the technology for meeting with standards is not available, and late last month Chrysler requested such an extension. The EPA's decision must be made by August 29.

flights can be fitted into NASA's budget. He also pointed out that the lab will be a unique capability of the shuttle, and thus direct cost comparisons with missions using conventional launchers are difficult to make. One possible extension of NASA's reasoning is that if the shuttle is used to carry out many sortie missions, there will be less justification for building a space station, which will also free more money in the 1980s for other missions.

Although NASA's new estimates have made the GAO's investigation of the economic arguments for the shuttle redundant in some respects, the accounting office's report contains much information which will undoubtedly be used to good effect by the project's critics. In short, the GAO concludes that it "is not certain that the space shuttle is economically justified . . . even though NASA's calculations show that it is". The report has reservations about possible cost overruns in development of the shuttle, and, more important, uncertainties in the overall size of the space programme.

In particular, the GAO points out that if there is an austere space budget so that there are many fewer shuttle flights than planned, development costs of the shuttle would not be spread so thinly, and the economic balance may be tipped in favour of expendable launchers. The report notes in several places that uncertainties in shuttle costs suggest that the choice of launcher system "should not be based principally on cost estimates".

Only in one instance does the GAO come up with a figure that is radically different from NASA's analysis, however. NASA officials have suggested in testimony before Congressional committees that the shuttle will be able to put a pound of payload into orbit for only \$160, compared with between \$900 and \$5,200 with expendable launchers. But the comparison is "not a meaningful one", the accounting office suggests, because NASA neglected to include the cost of development and production of the shuttle, and also computed the figure on the assumption that the shuttle would be used to maximum capacity. If corrections are made for those factors, the GAO suggests that the launch costs per pound are nearly \$3,500.

The GAO report and NASA's new mission model are sure to generate considerable controversy, and cloud the issue even further. But there are signs that Congressional opponents of the shuttle are beginning to use more straightforward arguments that go to the heart of the project. During hearings earlier this year before the Senate Committee on Aeronautics and Space Science, and the Senate Appropriations subcommittee that deals with NASA's

budget, Senator James Abourezk and Senator Proxmire have been asking some sticky questions about the short term effect of shuttle development on other items in NASA's budget.

This year, NASA is spending \$200 million on shuttle development. Next year the figure is expected to rise to \$475 million, and by 1977 it is expected to reach nearly \$1,200 million. Critics of the project want to know what effect that growth will have on NASA's space science and applications programmes. The crux of the matter is whether the Office of Management and Budget will allow the agency's budget to recover from the decline which has set in during the past few years. NASA officials say that they are confident that they will soon get back to the \$3,400 million level, but its critics say that there is no such guarantee.

The fact is that OMB allowed NASA only about \$3,100 million for 1974, and the projections for 1975 which were published with the 1974 budget request allow for only a modest increase to \$3,160 million. If the agency's budget stays at that level, critics of the shuttle have been suggesting that the science, technology and applications programmes will clearly suffer.

Whatever the force of those arguments, however, it is unlikely that the project will be stopped by Congress this year. Already the House of Representatives has easily turned back an attempt to delete funding from the shuttle from the NASA authorizations bill, and even the chief critics in the Senate are not optimistic about their chances. For one thing, they could only garner 21 votes last year against the shuttle, and that was the crucial vote which allowed NASA to go ahead and let contracts for the project. Another reason why the shuttle will be hard to stop is that the spectacular success of the improvised repairs to Skylab have at least demonstrated that man has some use in space. NASA will be sure to use that argument with telling effect in support of the manned space flight programme.

BIOMEDICAL RESEARCH

More Protests

NINE Nobel laureates, backed by the 6,000 members of the Federation of American Scientists and 2,000 other scientists last week sent a letter to President Nixon protesting at cutbacks in funding for biomedical research. The letter indicated two "fundamental complaints" about the Administration's proposed budget for 1974, namely the fact that increases in the budgets of the National Cancer Institute and the National Heart and Lung Institute are being offset by cutbacks elsewhere, and the decision to phase out NIH training

grants and fellowships. Those complaints are by now familiar, for the biomedical community has kept up a barrage of protests since the Administration's budget was unveiled in January.

The letter does point out, however, that in the face of financial stringency in all parts of the federal budget, funding for biomedical research cannot be expected to increase rapidly in the next few years, but adds that "we have a right to expect that the funds which are available to our discipline are allocated in a sensible way".

VENUS

Still Planning to Go

by our Washington Correspondent

IN spite of two setbacks, NASA officials are pushing ahead with plans to study Venus. A team of investigators has been chosen to provide experiments for a Pioneer spacecraft which, if all goes well, will send four probes into the Venusian atmosphere in 1978, and plans are also being drawn up to send an orbiter to the planet in the same year. In addition, there is another launch window in 1980 which will probably be used to launch a second orbiter or, if the 1978 probe mission fails, a second set of probes.

Those plans are, however, less ambitious than the programme that was being talked about last year, when NASA was hoping to send two sets of probes to Venus in 1976, an orbiter in 1978 and another orbiter in 1980. The first to be dropped was one probe mission which went by the board in November during bargaining between NASA officials and the Office of Management and Budget over the agency's 1973-74 budget (see *Nature*, 240, 177; 1972). But OMB officials still refused to allow NASA to put in for the money, and the 1976 launch has slipped to 1978. The second setback came earlier this year, when the European Space Research Organization decided not to join in the orbiter mission because of the uncertainties about US funding.

The 1978 probe mission will be launched in May, and arrive at the planet in December of that year. It will send one large probe, carrying about 60 pounds of instruments, and three small probes each carrying three pounds into the atmosphere. The large probe will take about 90 minutes and the small probes about 75 minutes to reach the surface, when their data transmission will stop. As for the orbiter mission, scientific experiments will be chosen in January 1974, and although ESRO has opted out of the mission, NASA officials are exploring the possibility that Britain or the Federal Republic of Germany will join in.

NEWS AND VIEWS

Vaccination Policy for Smallpox

FEAR of smallpox has again renewed controversy on the policy of routine vaccination against this infection in non-endemic areas such as the United Kingdom. Smallpox ceased to be endemic in this country in 1935 and all but two outbreaks, including the recent outbreak which presumably originated in a laboratory at the London School of Hygiene and Tropical Medicine, have arisen from importations. Indeed, since 1950 there have been 378 cases of smallpox in the United Kingdom resulting in 60 deaths. Alarming as these figures appear, the risks of vaccination complications may nonetheless outweigh the benefits of routine vaccination in non-endemic situations. Thus between 1951 and 1970 there were about 100 deaths from smallpox vaccination in England and Wales (Dick, *Brit. med. J.*, **3**, 163; 1971). Even so, these figures may be incomplete because there is probably some under-reporting, and non-fatal complications have been excluded. This is still perhaps not the best way to balance the policy of universal vaccination, the risk of smallpox and the complications of the vaccination procedure itself.

Herd immunity is still a common philosophy, and there is little doubt that a vaccinated community has an advantage, as far as mortality rate is concerned, over an unvaccinated community. It has been estimated, however, that in 1947, even after allowing for mass vaccination during the war and endemic variola minor during the period 1920 to 1934, the total immunity in England and Wales was less than 20% (Dixon, *Smallpox*, Churchill, London, 1962, p. 342). The acceptance rate for primary infant vaccination has been perhaps 40% and for re-vaccination probably less than 10%. In order to establish a high herd immunity it would be essential to have successful universal revaccination, every 3 years, throughout life. But even apart from the risks of vaccination it is difficult to justify immunization against a disease which has been successfully eradicated from all but seven countries—Bangladesh, Botswana, Ethiopia, India, Nepal, Pakistan and Sudan (*World Health*, October 1972). Because of the success of the eradication programme of the World Health Organization and the concomitant decline in the risk of smallpox importation, the United States and the United Kingdom discontinued in 1971 the practice of routine vaccination.

Severe outbreaks of smallpox from importations have been controlled for many years now by isolating cases, and tracing, vaccination and surveillance of known probable contacts. This policy of ring vaccination has been remarkably effective. There is, therefore, no place for mass unselective vaccination during a limited outbreak. Following the recent London incident some informed newspapers attempted to discourage mass vaccination, unlike the situation accompanying the outbreak of smallpox in South Wales in 1962, when at least eighteen people died from indiscriminate vaccination (Dick, *loc cit.*), generated to a large extent by panic.

At the same time it is imperative to vaccinate and revaccinate and to ensure successful vaccination of high-

risk groups such as doctors, nurses, laboratory workers, ambulance drivers and other hospital and public health personnel. In Europe during the period 1950 to 1971, for example, smallpox killed, among the hospital staff, an alarmingly high proportion of its young adult victims, fourteen or 70% of the twenty without previous vaccination (Mack, *J. infect. Dis.*, **125**, 161; 1972). The policy of selective and ring vaccination must continue, therefore, at least for the time being.

From our Medical Virology Correspondent

Diamond and Cavitation

WHY are diamonds found naturally in geological circumstances which belie the high pressures needed to synthesize them? This is the question which Galimov considers on page 389 of this issue of *Nature*. He comes to the interesting conclusion that diamonds could form when cavitation bubbles in flowing kimberlite magma collapse. The point is that cavitation can occur when the pressure on a liquid with gas dissolved in it is reduced below some critical value. Cavitation can take place, for example, in the vicinity of propeller blades on ships and in those circumstances can cause considerable damage.

Kimberlite usually occurs in vertical pipes a few tens of metres in diameter and a few kilometres long. Galimov calculates that magma flowing at a pressure of 20 kbar or so could be induced to cavitate if the magma passed through a constriction the cross-section of which is only two to three times smaller than that of the neighbouring regions of pipe. If one applies the celebrated Bernoulli relation it turns out that the pressure in these circumstances is reduced below that required for complete dissolution of carbon dioxide at 1,000° C. Further calculations reveal that the pressures and temperatures reached locally when cavitation bubbles collapse would be such as to allow the synthesis of diamonds.

Galimov also deals with the question whether the extreme conditions that exist when a cavitation bubble collapses last for long enough for a diamond to form. Extrapolating from the conditions in which diamonds have been artificially created in explosions, he calculates that appropriate natural conditions probably exist for a few milliseconds at a time, three orders of magnitude longer than is the case in a typical explosion experiment.

The cavitation concept also explains why some diamonds seem to have grown in a series of discrete steps: a newly formed diamond would evidently be a good site for the formation of subsequent cavitation bubbles and during each collapse the diamond would increase in size. And because the high pressures only occur very locally, other minerals usually formed in such conditions are not found in kimberlite.

CARCINOGENESIS

Testing Time for Toxicologists

by our Special Correspondent

A CHALLENGE has been thrown out to toxicologists and cancer researchers by the growing demands that new and existing drugs should be tested for carcinogenicity. At present the resources of materials and techniques available to them are limited and they must quickly look for new and improved methods. Taking up the challenge, the Drug Research Board of the US National Academy of Sciences called a conference in Washington DC on May 23–25 to consider carcinogenesis testing in the development of new drugs.

The essence of the procedures most commonly used to test for carcinogenesis has been to treat animals—usually rats and mice—with the drug for varying times, to look for effects on the time of appearance and the number and types of tumours that develop. There are, however, many problems. For example, what strain of animal should be used; how much drug should it receive for how long; and what diet should it eat while being dosed? These, and all the other questions tackled at the meeting, were first thrashed out during day-long discussion groups, whose leaders reported their recommendations during the second and third days.

Dr M. B. Shimkin (University of California, San Diego) recommended, on behalf of his group, that inbred strains of mice provide the best single animal for testing, but that rats are also desirable for this purpose, with the Syrian hamster probably the next best species. Larger animals, including primates, however, are impractical for bioassays and should be restricted to special problems. Nevertheless, the use of more non-rodent species should be explored.

The group concerned with the size of doses recommended, through its leader Dr L. Friedman (Food and Drug Administration), the use of the maximum dose that permits valid interpretation of the experimental observations. Usually, the group decided, with rodents this dose should be such that it will not depress the increase in weight more than 10% compared with controls. This dose should also leave 50% of treated animals alive at the end of the study and produce no pharmacological effects to invalidate the results. Two other doses should be used, however, the second highest usually being 10–50% of the maximum. Dr Friedman's group recommended that treatment, which should begin at conception and continue until completion of the study, or death, should be given on five to seven days each week. The route of administration to the animals should be oral or

topical when they apply to human use of the drug. Intramuscular and subcutaneous injection should be used only if absorption is poor in the test animal after oral administration, and inhalation should be limited because animal models are not sufficiently established to yield valid negative results with substances of unknown carcinogenic potency.

Diet can affect considerably the progress of carcinogenesis in rodents, and so the choice of food for test animals is crucial, as Dr P. M. Newberne (Massachusetts Institute of Technology) reported. His group recommended that diets for carcinogenesis testing should be standardized, so that results from different laboratories can be compared and reproduced. For this purpose they would like to see manufacturers producing semi-synthetic diets free of known enzyme inhibitors and antioxidants, and containing standardized amounts of protein, carbohydrates, vitamins and minerals.

Some of the problems besetting the hard-pressed pathologist who has to interpret the results of these various carcinogenesis tests were described by Dr W. Richter (University of Chicago), giving one of the individual contributions to the conference. Sometimes a supposed tumour turns out to be an abscess, or there are cases such as the hyperplastic rodent bladder which looks, in the microscope, like a normal primate bladder.

Considerable energy is now being devoted to the development of tests which can be carried out more quickly than those involving live animals. Dr J. A. DiPaulo (National Institutes of Health) said that some tests using a Syrian hamster cell culture system at the National Cancer Institute can be completed in ten days. Speaking for the discussion group concerned with tissue culture techniques, Dr B. Weinstein (Columbia University, New York) said that modesty is required in anticipating the outcome of tests of carcinogenicity on, as well as hamster cells, mouse fibroblasts and epithelial cells, which come principally from the rat liver. The problems of using tissue of human origin also seem to be legion, as the discussion group charged with considering this question discovered. Dr A. Freeman (Children's Hospital, Akron, Ohio) reported the recommendations that organ culture methods must be developed for testing carcinogens, and that cell banks should be established. His group also suggested that a committee should be set up to help carcinogenesis testers to obtain human tissue from inbred populations, which may be

predisposed to cancer, in remote geographical areas. When it comes to obtaining the necessary evidence from the transplantation of transformed cells into donors (to establish whether they develop cancer), there are obvious objections to the use of human subjects. Dr Freeman's group therefore recommended that evidence should be accepted from xenografts, but only with concomitant and careful pathological investigation.

The general feeling of the conference, summarized by Dr D. T. Rall (National Institute of Health Sciences), was that the life-time studies of whole animals are essential to carcinogenesis testing, but that short term methods also have their function.

MEMBRANE TRANSPORT

Transduction Deductions

from our Molecular Biology Correspondent

THE study of active transport is a thriving industry at the heart of which lies a kernel of profound ignorance: it is simply not known how the hydrolysis of ATP occasions the transfer of ions through the membrane against a concentration gradient. Many facts about the process have been amassed, one of the most salient of which emerged only recently, namely that the calcium ATPase of sarcoplasmic reticulum at least can be made to go into reverse, to function, so to speak, as a motor instead of a dynamo, and synthesize ATP from ADP and phosphate, when the concentration gradient is allowed to run down. A facet which may be common to all ATPase systems is the ability to catalyse the exchange of oxygen between water and phosphate, which, it is supposed, is in some way related to a step in the ATPase mechanism. Boyer and his associates have shed some light on this dark corner, and two new articles on ATPases of quite different kinds again point to an underlying unity.

Dahms and Boyer (*J. biol. Chem.*, **248**, 3155; 1973) have examined the sodium-potassium ATPase from kidney membrane and from the electroplax of the electric eel. The preparations of these enzymes catalyse the rapid entry of ^{18}O from isotopically enriched water into phosphate ions in the medium. This is clearly related to the primary enzymatic function, for it requires potassium and is annihilated by sodium, and also ouabain, which is a specific inhibitor of sodium-potassium ATPase. It also requires the presence of magnesium. It is unaffected, on the other hand, by oxidative phosphorylation uncoupling agents, such as dinitrophenol, and it differs also from the mitochondrial system in requiring no nucleotide. Moreover there is no exchange between ATP and either water or orthophosphate.

These features render it unlikely that the water-phosphate exchange is a result of reversal of the ATPase reaction. On the other hand, the exchange reaction, when inhibited by sodium, can be set going again by addition of ATP. This points to a phosphorylation step in the mechanism, and there is also evidence that ouabain operates by stabilizing an acyl phosphate intermediate of the enzyme.

The inferences are supported by the second study (Kanazawa and Boyer, *ibid.*, 3163), which concerns the calcium-magnesium ATPase of sarcoplasmic reticulum. This enzyme stimulates the same exchange of oxygen between water and phosphate in the absence of calcium, which is a strong inhibitor. Neither ouabain nor oxidative phosphorylation uncouplers affect the reaction. The turnover efficiency is greater than that of the calcium ATPase reaction by more than an order of magnitude. The calcium-dependence is strongly cooperative, and the inhibition can be reversed by chasing off the calcium with high magnesium concentrations. Exchange is inhibited by ATP and by ADP. Again, the exchange reaction can be linked to phosphorylation of the enzyme, for under the same conditions phosphorus from orthophosphate is incorporated into the protein. Calcium exerts a strongly cooperative control over this, as over the exchange reaction. These observations, it should be noted, pertain to sarcoplasmic reticulum vesicles. A monionic detergent, which leaves the ATPase activity unimpaired, stops both ion transport in the vesicles, and the oxygen exchange reaction, to which the process is evidently related.

On the basis of these results, Kanazawa and Boyer have constructed an active transport scheme, involving a conformational "pre-equilibrium" of the enzyme, with an allosteric response to calcium, for which the existence of two sites per molecule is surmised. In the state of high calcium affinity the enzyme is responsible for translocation of calcium ions. The other conformational isomer has a single magnesium site (because there is no cooperative response to magnesium), and does not bind calcium, and is responsible for moving magnesium ions across the membrane. The transport cycle contains phosphorylation and dephosphorylation steps, and the exchange of the oxygen can be accommodated in the scheme as a consequence of reversal of the reactions involving the uptake of phosphate in the presence of magnesium, and the covalent coupling of the phosphate group to a side chain, with elimination of water. The exchange reaction may thus prove to be the key to the model for active transport and related energy transduction processes, and the implications that Kanazawa and Boyer have

found in it will now need to be digested by biochemists at large.

An at least superficially simpler system is the glucose transport machinery of red cells. This has served therefore as something of an archetype for pencil-and-paper models of active and passive transport. The directness of some observations and their interpretation by Edwards (*Biochim. biophys. Acta*, **307**, 415; 1973) on this system comes as an agreeable anodyne after the alarms and excursions of ion pumps. It is known that glucose transport is inactivated by exposure of the red cells to the amino group-specific reagent, fluorodinitrobenzene. What Edwards now finds is that the rate of inactivation, which must be a reflexion of the conformation or accessibility of the target protein, is modified by the presence of glucose, and differs by more than two-fold according to whether the glucose is inside or outside the cell. The regulation of the reaction is specific for glucose, and is not simply an osmotic phenomenon. The results are most easily explained in terms of two alternative states for the glucose receptors, which, Edwards suggests, may be the outward and inward-facing orientations of a carrier protein.

MARINE BIOLOGY

Fish Eggs and Larvae

from a Correspondent

A SYMPOSIUM on the early life history of fish, sponsored by a number of international organizations including FAO, ICES (the International Council for the Exploration of the Sea), SCOR (the Scientific Committee for Oceanic Research) and IABO (the International Association of Biological Oceanography) was held at the Dunstaffnage Marine Research Laboratory of the Scottish Marine Biological Association, Oban,

Argyll, Scotland, from May 17 to 23.

The meeting generally accepted that high mortalities in the early stages are an important factor in determining the future brood strength and success of a year-class on recruitment to the subsequent fishery. From the results of plankton surveys on fish eggs an extensive mortality seems to occur in some species, like sole (J. D. Riley, Fisheries Laboratories, Lowestoft) and pilchard (A. J. Southward and N. Demir, Marine Laboratory, Plymouth), which cannot be explained by current systems carrying them out of the area, nor by predation. Indeed, in the case of buoyant eggs dead specimens are commonly found in the plankton hauls. It seems that in the developmental process there may be imperfections which could vary from year to year, which could be caused by maternal effects, and which could be enhanced by unfavourable environmental conditions. The extent to which eggs in delicate stages of development, for example before gastrulation, could be killed during capture has obviously been inadequately investigated and needs further study. After hatching, losses due to starvation and predation are heavy. Many larvae are extremely small and need food organisms of less than 10 μ in size (B. R. de Mendiola, Institut del Mar, Lima). Attempts to quantify the nutritional status of larvae, and therefore their viability, have been attempted in the laboratory by using chemical and morphological techniques. There is some evidence that starving larvae, which are inactive and near neutral buoyancy, may be selectively sampled by plankton nets (J. H. S. Blaxter and K. F. Ehrlich, Dunstaffnage Laboratory). Predation has been studied experimentally and it is likely that unsuccessful strikes by predators may cause additional mortality because of the

ϕ X174 Promoter Regions Isolated

By using bacterial restriction endonucleases to cleave viral DNA molecules into sets of fragments that can be ordered molecular virologists can now obtain physical maps of viral genomes. At the same time, by using ribosomes or DNA dependent RNA polymerase to protect respectively ribosome binding sites or polymerase binding sites from nuclease attack, it has proved possible to isolate these interesting regions of various nucleic acids and a few such fragments have been sequenced. In *Nature New Biology* next Wednesday (June 20) Chen, Hutchison and Edgell report how they have combined these two techniques to isolate and localize the promoter regions, or to be more precise the RNA polymerase binding sites, of coliphage ϕ X174 DNA.

Having previously analysed and

ordered the fragments produced by the digestion of double stranded replicative form ϕ X174 DNA with three endonucleases, Chen and his colleagues assayed the extent to which these various fragments bind RNA polymerase. They also bound RNA polymerase to intact ϕ X174 DNA, digested the DNA with pancreatic DNase and isolated the pieces of DNA protected by the polymerase. These fragments were then identified by hybridizing them to fragments of ϕ X174 DNA obtained with restriction enzymes.

The results of these and other experiments indicate that the ϕ X174 genome has three ribosome binding sites which may not be identical. No doubt Chen *et al.* will now begin to sequence these fragments which presumably code for the initiation of transcription.

delicate integument and general fragility of larvae.

There is an almost complete lack of data on microdistribution. Most plankton nets are designed to filter large volumes of water and are not always equipped with closing devices to limit the depth of sampling. There is indirect evidence that larvae make small scale horizontal and vertical migrations and are not completely dependent on convection currents. Concentrations of larvae may be found in certain areas which suggest some sort of positive aggregating mechanism (J. M. Miller, Hawaii Institute of Marine Biology). In experimental conditions anchovy larvae will aggregate on small food patches (J. Hunter and G. Thomas, Southwest Fisheries Center, La Jolla, California) and herring larvae will perform vertical migrations which seem to have no obvious immediate adaptive value except in exploiting current systems at different levels in the water (S. Seliverstov, Polar Research Institute, Murmansk). A reshaping of ideas on larval distribution may emerge from the symposium.

Substantial knowledge on tolerance to high temperature, low oxygen, H_2S , heavy metals and other potentially deleterious factors in the environment has been accumulating in the past few years. These data are of value for assessing the losses due to entrainment in power station effluents (D. Hoss *et al.*, Atlantic Estuarine Fisheries Center, Beaufort, North Carolina) and for assessing the possibilities of exploiting heated effluents to improve larval growth in aquaculture. The improvement in culture techniques in the past few years has been remarkable, especially due to work in the United Kingdom, France and the United States. New sources of small food organisms and improvements in technique mean that, given adequate finance, most fish species can be reared from the egg. The main remaining stumbling block is obtaining mature adult fish. Although some species with extended spawning periods, such as the anchovy, can be induced to mature by the use of suitable photoperiods and temperature regimes, in most species the control of the maturation process is not practised, although spawning in ripe adults can be induced by injection of gonadotropins. In the symposium, Pütter's theory was once again resuscitated by Dr J. Flüchter (Bayrische Landanstalt für Fischerei, Starnberg) and it must be admitted that the possible use of dissolved organic matter as larval food has still not been resolved. In aquaculture the use of hybrids and gynogenesis is gradually coming into vogue (C. E. Purdon, Fisheries Laboratory, Lowestoft) and the manipulation of genetic material and selective breeding are obviously destined to be profitable lines of research in the future.

GRASSLAND ECOLOGY

Land Use in the Veld

from our Plant Ecology Correspondent

It is now widely accepted that much of the grassland and savannah of Africa is not climatic climax vegetation. The combined effects of regular firing and intensive cattle grazing have imposed upon these habitats a powerful constraint which has deflected the natural course of succession. It is a matter of speculation whether these areas could have developed naturally in this way, for example, as a result of fires from lightning strikes, in the absence of human influence.

Strang (*Biol. Conserv.*, 5, 96; 1973) now describes the results of "bush-encroachment" which is currently causing problems on the high veld of Rhodesia. In this upland area the rainfall (75–105 cm) is adequate for the support of woodland and the region can be regarded as a transition zone between dry savannah to the south and forest to the north. The early European settlers of this region, however, found extensive grasslands which were maintained by

frequent fires and high grazing pressures from domesticated cattle. Land use in the high veld has changed little since those days, for only about 15% of the area has proved suitable for arable agriculture. More recently, however, the intensity and frequency of fires have been reduced very considerably and this, when combined with overgrazing which is becoming increasingly common, may be the chief factor underlying the spread of scrub and woodland.

The encroachment of scrub reduces foliage production in the grasses, and is therefore detrimental to the cattle industry. Experimental clearance of this woodland has been shown to increase the carrying capacity of the veld from one beast per 8–12 ha to one beast per 3–4 ha. Strang evaluates the various methods available for scrub control and clearance and concludes that the re-introduction of intensive burning would not be an efficient means of attaining these ends. If burning takes place just once every 4 years then it effectively means that one-quarter of the grazing lands will be non-productive in any given year. The alternative methods of control, such as manual clearance or

Haemin Effect on Protein Synthesis is Non-specific

THE conclusion reached by Mathews *et al.* in their study of the specificity of the control of protein synthesis by haemin (see *Nature New Biology* next Wednesday, June 20) was foreshadowed by the results of an earlier study by Mathews in which he showed that haemin stimulates the translation of endogenous messenger RNA and EMC viral RNA, as well as globin RNA, in the ascites sub-cellular system.

The non-specific effect of haemin is even more dramatically demonstrated by this new work in which Mathews *et al.* used the rabbit reticulocyte lysate as a test system and ^{35}S -methionine to label the nascent protein.

Mathews *et al.* show that not only rabbit globin but also the chiefly larger and presumably haemin-free reticulocyte proteins synthesized in small amounts (about 10% relative to globin) in the endogenous system (lacking added mRNA) are stimulated by haemin. There is a gradient of stimulatory effect from low to high molecular weight proteins for which Mathews *et al.* propose the explanation that completion of existing polypeptide chains contributes significantly to the pool of nascent proteins analysed.

Messenger-directed synthesis of mouse globin, α and β calf lens crystallin, and EMC viral proteins in this system are all stimulated markedly by haemin. It has been suggested that haemin prevents the action of one or two different inhibitors of protein synthesis

which accumulate in the lysate during incubation. Furthermore, the Cambridge group have shown previously that both the inhibitors act by preventing the association of met-tRNA_f with the small ribosomal subunit before binding mRNA. In such a case it would not be surprising if the effect on translation of mRNA were non-specific. The experiments presented in *Nature New Biology* provide a good test of the suggested mechanism of action of the inhibitors, and the new results are fully consistent with this effect. Mathews *et al.* cite an article in the press in which they claim that the met-tRNA_f-40S ribosome complex is a true intermediate in the initiation of protein synthesis.

Mathews *et al.* have thus exploded the long-standing myth that haemin (which is incorporated into haemoglobin) has a specific regulatory effect on globin synthesis. Previous workers in this field failed to carry out the appropriate control experiments before propounding this theory. The question remains whether the haemin effect has any physiological significance because, as Mathews *et al.* point out, evidence has been adduced to show that protein synthesis in HeLa cells (unlike that in reticulocytes) is resistant to inhibition by iron chelating agents. They suggest that the haemin effect, even if it does not operate *in vivo*, may be a "paradigm" for the control of protein synthesis when a general mechanism of switching on or off is required by the cell.

ring-barking, mechanical clearance and the use of arboricides, are all expensive. Strang favours an alternative to cattle ranching, that is the cropping of wild herbivores.

Game ranching offers a number of advantages over cattle grazing. In particular the wild herbivores are able to exploit the full spectrum of natural primary production rather than being dependent on certain palatable species. Most of the game animals involved are browsers rather than grazers, hence they would not be adversely affected by scrub encroachment. An important argument against this solution is the difficulty of disease control in mixed communities of wild herbivores. Perhaps a greater problem is the social upheaval which would undoubtedly result from such a radical agricultural policy—a policy which has yet to be justified economically.

ENERGY RESERVES

Bio-fuel Gauge

from a Correspondent

OF all the problems facing small homoiothermic animals living in cold places, hypothermia is possibly the greatest. This is probably one of the reasons why there is an abundance of small birds and mammals in the tropics and a scarcity in Arctic and Alpine regions. Hypothermic torpor in small animals is far from uncommon, and recently it has been shown to occur in the broad-tailed hummingbird (*Selasphorus platycercus*) of North America. What is especially interesting is the series of factors bringing about its onset.

Calder and Booser (*Science*, **180**, 751; 1973) have examined the fluctuations in temperature of hummingbird nests during incubation in the nesting seasons of 1971 and 1972. A study site at 2,900 m elevation was established in the Elk Mountains, Gunnison County, Colorado. Thermistor probes were embedded in elastomer "eggs", and the pattern of the hen's absences from the nest was recorded by interpretation of the rapid cooling spikes on the temperature trace.

Hummingbirds cannot feed in the rain, and during one particular 24-h period in June 1972 the weather deteriorated to such an extent that the incubating females missed several of the normal, periodic feeding flights. The hens of two wired-up nests lost approximately 21 and 12% respectively of their normal feeding opportunities. In an animal with a delicately balanced energy budget this reduction in energy intake can be expected to have a profound effect on subsequent behaviour. At about 0100 h in the night following this catastrophe the air temperature fell to -1.0°C , and the temperature of nest 1 began a steady decline from 31°C to 6.5°C . It then

stabilized and seemed to be regulated. The temperature of nest 2 showed a similar decline and stabilized at the same level, but the decline started almost 3 h later. In both nests normal temperature was regained by 0500 h—the time at which feeding flights normally recommenced.

The chart from nest 2 showed several sharp drops immediately before the onset of hypothermia. This recalls the "test drops" made by ground squirrels when entering hibernation (Sturmwasser, *Am. J. Physiol.*, **196**, 8; 1959), but they also resemble normal brief nest departures. It is therefore possible that the hen from nest 2, sensing its energy reserves were insufficient for the weather conditions, tried to feed during the night in an attempt to preclude the need for torpor.

The fascination of these observations lies in the fact that the periods of hypothermia endured by the two hens started at different times but ended simultaneously. The length of time spent in torpor seemed to be correlated with the reduction in feeding opportunities (21 and 12%) presented to hens in the previous daytime. This is not so strange, for it has long been known that the duration of torpor induced in laboratory rodents is correlated with food intake (Bartholo-

mew, *Ecology*, **50**, 705; 1969). What is curious is that the reduction in number of occasions for energy intake acts as a fuel gauge to "inform" the bird of the precise moment when it should enforce the necessary economies. These economies seem to be withheld until the last possible moment, doubtless in the hope of a sudden rise in air temperature.

Cooling seems not to damage birds' eggs, but it does increase incubation time. Perhaps frequent resort to torpor is an important factor in the long incubation periods seen in hummingbirds nesting high in the Andes.

MATERIALS SCIENCE

Analysis of Surfaces

from our Materials Science Correspondent

ARGUABLY the most productive new instrument in materials science since the war has been the electron microprobe analyser. This instrument, which depends on the emission and analysis of characteristic X-rays, stimulated by a minute exploring beam of electrons impinging on a selected point on a polished specimen, has transformed the study of solidification, phase transformations, diffusion and corrosion. Its essential feature is that the chemical ana-

How Do Nodules Grow in Freshwater?

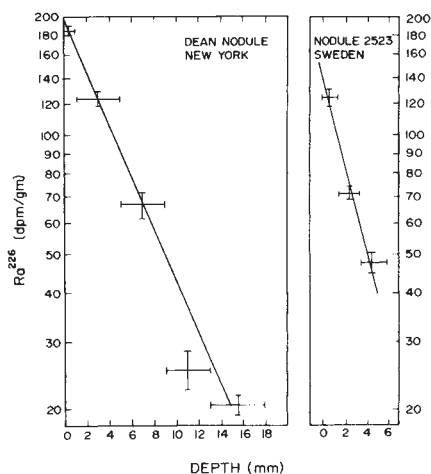
THE question how ferromanganese nodules grow in freshwater is considered by Krishnaswami and Moore in *Nature Physical Science* next Monday (June 18). They discuss the insight cast on this problem by their study of two nodules, one a sphere 2 cm in diameter from Lake Alstern, Sweden, and the other a saucer shaped concretion about 2 cm thick from Oneida Lake, New York.

If the growth rates of the deposits are in the range 100 to 1,000 mm per

10^3 yr, ^{226}Ra (half life 6.7 yr) and ^{210}Pb (half life 22.3 yr) should be ideal for measuring the rate of accretion. Surprisingly, however, the appropriate analysis of these two nodules yields accretion rates of only 1 to 3 mm per 10^3 yr, with ages around 6,500 yr, similar to those reported for iron-manganese nodules from Lake George, New York.

Taken at face value, then, these data suggest that sediment around the nodules accumulates 1,000 times faster than the nodules grow. How, then, do the nodules maintain their position at the sediment/water interface? Perhaps active currents play a part in keeping the nodules exposed, which would also explain why nodules are abundant where wave action is strong and fine sediment absent. Or perhaps there is another interpretation.

Krishnaswami and Moore point to three possible sources of error in their approach: first, the ^{226}Ra concentration in the lakes may be increasing; second, radium may migrate to the outside of the nodules during growth (although no such effect is observed for barium); and third, ^{226}Ra may be diffusing inwards, producing an apparent radioactive decay with depth. But if the nodules are as old as they seem to be, they should certainly provide valuable information on the trace element history of the water in which they formed.



Variation of ^{226}Ra with depth in the two nodules.

lysis is localized, whereas X-ray fluorescence analysis of the longer established kind, which depends on excitation by hard incident X-rays, averages the composition over several square centimetres. The wheel is, however, coming full circle with the introduction of macroscopic X-ray analysis by electron excitation, which in its latest form allows measurements of elements down to boron (Price, *Metals and Materials*, 7, 140; 1973). The use of greatly improved detectors in conjunction with non-dispersive energy analysis has steadily improved sensitivity as well as light-element detection in both microscopic and macroscopic variants of electron-excited X-ray analysis.

Electron probe microanalysis used to be regarded as a form of surface analysis, but in fact the effective depth over which composition is integrated is of the order of $1\ \mu\text{m}$. True surface analysis, by contrast, requires physical methods that explore a single surface monolayer, or $\sim 1\ \text{nm}$ depth at most. There is now a very varied array of techniques available—the emission of photons, electrons, ions or neutral particles can be stimulated by any of these four, or by heat or an intense electric field for good measure.

More than twenty permutations of stimulation/emission exist, each with its own distinct physical theory, sensitivity, speed, depth of measurement, convenience and limitations. Electron-excited X-ray (that is, photon) emission is merely one among many. The onlooker who is concerned with using these techniques rather than developing them may be forgiven for being bemused. Most survey articles, of which there is no shortage, fasten on one technique to the exclusion of all others. For this reason, a new survey entitled "New Developments in the Surface Analysis of Solids" does special credit to the author, Benninghoven of the University of Cologne (*Appl. Phys.*, 1, 3; 1973). (The journal is a new English-language venture by Springer-Verlag which replaces the excellent German-language *Zeitschrift für angewandte Physik*, which ceased publication at the end of 1971.)

Benninghoven is a specialist in surface studies and his name is especially associated with secondary-ion mass spectrometry (SIMS), of all methods the one best suited to analyse a single surface monolayer. He describes in some detail in his survey the advantages of SIMS (including the ability to follow chemical reactions at the surface as they progress), but devotes attention also to the various forms of secondary-electron spectroscopy (including both Auger electron (AES) and photoelectron spectroscopy, the latter usually known by its originator's designation of ESCA—electron spectroscopy for chemical

analysis), and even to the very novel technique of "soft X-ray appearance potential spectroscopy", which depends on the sudden appearance of characteristic X-ray emission lines as the energy of bombarding electrons is gradually increased. Benninghoven explains the essential physics of each technique without getting side-tracked along fascinating but inessential detours (such as the mechanism of surface-ion emission in the sputtering situation) and shows how emission of different types can be reliably distinguished, how the state of chemical combination can be assessed by some techniques but not by others, how and why the effective depth of analysis varies between techniques, which are best for analysing light elements including hydrogen, and how sensitivity varies. The examination of states of chemical combination is particularly important in many applications, but the very power of this technique (see Siegbahn, *Endeavour*, 32, 51; May 1973) has led to its use predominantly in

"bulk situations" rather than in surface studies hitherto.

Another technique, related to SIMS, is ion-scattering spectrometry (ISS). Here an exploring beam of incident low-energy ions, often He or Ar, is scattered from the surface layer: the energy distribution of the same ions is measured after scattering, and simple calculations based on Rutherford scattering theory permit deductions as to the masses of the atoms in the surface layer. This technique is outlined in a clearly and comprehensively written survey of trace analysis techniques for solids (both for surfaces and for bulk solids) by Kane and Larrabee (*Ann. Rev. Mat. Sci.*, 2, 33; 1972). This review can with benefit be read in association with Benninghoven's.

Overall, Benninghoven's most useful survey leaves the impression that SIMS, a technique not yet widely familiar, has particular promise as a highly sensitive and discriminating method of identifying and analysing surface species.

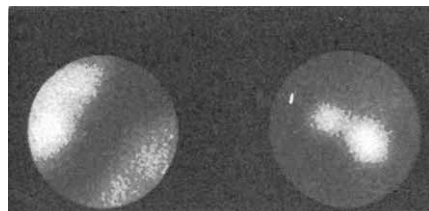
New Techniques for Ionospheric Research

THE simplest model of the ionosphere is one in which the electron concentration at a given height does not vary with position. It is a good first approximation in most situations, but for many years ionospheric workers have been considering the ways in which it fails to represent the real ionosphere; for example, spatial irregularities of various kinds are often found, and there have been attempts to study their structure and movements, often in the hope of obtaining information about winds or waves in the ionosphere.

Two Australian communications in *Nature Physical Science* next Monday (June 18) each describe a different improvement in technique to study such phenomena. Both use large arrays of aerials which can achieve high angular resolution in the reception of radio waves at frequencies from 2 to 6 MHz, but differ greatly in their method of handling the received signal. Briggs and Holmes use for each aerial a super-heterodyne receiver connected to a transducer which forms part of an acoustic array—an analogue of their radio receiving system—under water. If the acoustic sources lie on a plane surface they would simply produce an acoustic field analogous to the original electromagnetic field, and this is not immediately useful. The sources are, however, arranged on a spherical surface in such a way that a plane wave falling on the aerials gives rise to an acoustic wave focused on an image plane. The position of this focused point is a measure of the direction of arrival of the original radio wave. A set of transducers on this image plane

energizes an array of light-emitting diodes to make visible the directions of all incident radio waves.

The figure here shows on the left the distribution of radio wave intensity over the array and on the right the angular distribution obtained from it by the acoustic technique; two modes of propagation can be distinguished. The method can also be used to follow the reflexion points of waves from a fixed transmitter and thus to study irregularities. The transmitter need not be pulsed nor indeed controlled by the experimenter, so uses in radio astronomy and in direction-finding are quite possible.



The method of Brownlie *et al.* is more limited in application. A beam is formed by transmitting pulses from one arm of a cross and receiving them on the arm at right angles, and is steered by phase shifters under computer control. By rapid scanning a display can be produced showing the angular distribution of received radio waves. The technique is clearly very suitable to the study of highly irregular ionospheric structures such as "spread-F". Similar systems have been constructed previously for different purposes, but with receiving beams only (for example, Morris *et al.*, *Proc. Instn. elect. Engrs.*, 110, 1569; 1963).

EARTHQUAKE PREDICTION

Precursory V_p/V_s Changes

from our Geomagnetism Correspondent

MORE than a decade ago, Kondratenko and Nersesov (*Trudy Inst. Fiz. Zemli*, **25**, 130; 1962) reported that the velocity of crustal P waves increases after moderately large earthquakes in the Tadzhikistan region of southern central Asia. They had analysed more than 800 travel-time curves from foreshocks and aftershocks of earthquakes with magnitudes 5 or greater and occurring during the period 1958–1961, and had concluded that the average P wave velocity was 5.3 km s^{-1} prior to main shocks and 6.3 km s^{-1} afterwards. This was the first report of a variation in seismic velocity associated with earthquakes; but unfortunately it was not translated from the Russian and so apparently remained unknown in the west (and even more surprisingly in Japan) until Savarensky (*Tectonophysics*, **6**, 17; 1968) reviewed the data in English.

There can be little doubt that had this discovery been made known at the time outside the Soviet Union, it would have attracted considerable attention, especially in Japan where earthquake prediction research had long been a subject of great concern. The sheer magnitude (more than 15%) of the reported velocity change of the P waves would have ensured attempts to repeat the analysis elsewhere. Rikitake (*Earth Sci. Rev.*, **4**, 245; 1968), for example, was later to imply doubt about the validity of the Russian data on the grounds that a P wave velocity change of 15% would correspond to a 30% change in elasticity. As it was, the delay in the communication of the Russian results was compounded by the fact that, throughout the 1960s, seismic velocity was not apparently among the many parameters independently studied from the point of view of earthquake prediction.

Following Savarensky's report and other reports of Russian work (see, for example, Nersesov *et al.* in *The Physical Basis of Foreshocks*, Moscow, 1969), the subject was taken up by United States seismologists. In 1969, Semenov (*Izv. Acad. Sci. USSR Phys. Solid Earth*, **4**, 245; 1969) had shown that prior to earthquakes in the magnitude range 3–5, the P wave–S wave velocity ratio (V_p/V_s) decreased by about 6% and then increased again to about its normal value just before the onset of the main event. Aggarwal *et al.* (*Nature*, **241**, 101; 1973) then reported a similar phenomenon involving V_p/V_s decreases of up to 13% for earthquakes in New York in the magnitude range 1–3. But these changes all involved relatively small events; and it was clearly desirable to determine whether similar precursors apply to

larger earthquakes. Proof that they do has now been supplied by Whitcomb *et al.* (*Science*, **180**, 632; 1973) in respect of the 1971 San Fernando earthquake (magnitude 6.4). This report from Whitcomb and his colleagues, coming at a time when many people have become discouraged by the apparent lack of progress in the highly intractable problem of earthquake prediction, is likely to become a landmark in the subject, for not only does it present convincing evidence for the phenomenon concerned, but it has also been possible to view an earthquake precursor in terms of a realistic physical model.

The facts are easily stated. Whitcomb *et al.* have used P and S waves from nineteen earthquake sources during 1961–1970 to show that the V_p/V_s ratio in the San Fernando epicentral region had an average value of 1.75 up to 1967 but decreased suddenly by 10% in the middle of that year and then gradually increased to about its normal value at the time of the 1971 shock. When the P and S wave velocities are examined individually both are found to change in the same sense as the V_p/V_s ratio, but the V_p variation is the greater and the variation in V_s is little greater than the uncertainties of measurement. Thus, contrary to some previously stated beliefs, it is V_p which accounts for the change in V_p/V_s . This is an important result, not only because it leads directly to a possible physical explanation of the precursory variation but because of its practical consequences. Man-made explosions produce chiefly P waves, any S waves generated being difficult to measure. The new result thus opens the way to the use of artificial sources to detect precursory V_p/V_s changes, rather than having to depend on the vagaries of earthquakes.

The second important result concerns the interval between the sudden V_p/V_s decrease and the onset of the earthquake, which in the San Fernando case is 3.5 yr. Combining this result with data from previous studies, Whitcomb *et al.* show that, over the magnitude range 0–6.4 at least, the precursory interval increases logarithmically with earthquake magnitude. Extrapolation of this variation (the validity of which will need testing by future studies) suggests that a magnitude 7 event would have a precursory interval of 8 yr and that a magnitude 8 event would have an interval of 40 yr. Clearly no great reliance should be placed on these absolute figures at this stage; but what is quite certain is that the precursory interval increases with magnitude. By contrast, the precursory V_p/V_s decrease

is independent of magnitude.

Whitcomb and his colleagues then go on to show that all of these results may be accommodated, both qualitatively and quantitatively, by a physical model derived from the known behaviour of fluid-filled, porous media—in particular, the phenomenon of rock dilatancy, or the increase of volume resulting from a change in shape. In the field, the change in shape is thought to be caused by shear stresses associated with regional tectonic strain. Assuming the pores and cracks in the rock to be initially saturated with fluid, the dilatant volume increase will lead to a decrease in pore fluid pressure, and if the dilatancy is large enough the pore and crack volume will ultimately exceed the fluid volume. In this under-saturated stage the enlarged rock voids will partially contain vapour, resulting in a reduction of the overall bulk modulus. This produces a decrease in V_p , but little change in V_s and thus accounts for the drop in V_p/V_s .

The effect of the reduction in pore fluid pressure is to increase the fracture strength of the rock and thus delay the onset of the earthquake. Fluid flow from outside the dilatant volume, however, gradually returns the permeable rock to its saturated state, producing a gradual increase of V_p/V_s and a gradual decrease in fracture strength until an earthquake occurs. The actual variations in V_p and V_s during these processes depend on the range of velocities in the rock between the saturated and undersaturated states. This range is independent of earthquake magnitude, and so the drop in V_p/V_s must also be independent of earthquake magnitude. On the other hand, the rate at which the rock returns to the saturated state depends partly on the permeability of the rock and the availability of fluids but also on the dilatant volume. The dilatant volume, in turn, must depend on the amount of stored mechanical energy which presumably determines the magnitude of the final earthquake. The precursory interval—the time taken for V_p/V_s to return to its normal value—should thus depend on the earthquake magnitude, the greater the magnitude the longer being the interval.

It is this marriage of observation and physical theory which makes the report from Whitcomb and his colleagues particularly important. Thus, in spite of the historical accident which delayed the wider communication of the original discovery, the V_p/V_s precursor now emerges as probably the most promising of all possible earthquake precursors. As Whitcomb *et al.* point out, however, there are other phenomena (such as electrical conductivity) associated with dilatancy. Some of these have already been studied with a view to earthquake prediction, but such studies may now become more significant.

Contemporary Population Densities and Human Health

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High population densities are not associated with high mortality when socioeconomic factors are controlled.

HIGH population densities are commonly asserted to be one of the chief causes of crime, mental breakdown, stress-related illness, and other contemporary problems (for example, refs 1-3). This assertion has been based in part on studies of the social pathology that develops in caged rodents at high population densities (for example, refs 4 and 5). Although it may be intriguing to extrapolate from the behaviour of rats and mice physically trapped in a very simple and apparently boring⁶ cage to the behaviour of humans economically trapped in a decaying urban area, no scientific validity can be assumed for this extrapolation. We do not even know how high densities would affect rodents in complex environments that would provide the opportunities which natural environments provide for exploration and for avoiding aggressive interactions. Furthermore, the great differences between the behavioural repertoire, social structure, and physical surroundings of the rodents in the experiments and those of people in the cities make it unwarranted to extrapolate from rodents to humans on the basis of a single similarity: that both groups are living at densities many times higher than have been typical of their species.

Careful studies on humans have failed to provide convincing evidence that contemporary population densities have significant harmful effects. (Brief reviews and critiques may be found in refs 7-9.) There is no simple or reliable relationship between changes in population size and the frequency and intensity of wars¹⁰. Brief exposures to extremely high densities have a variety of effects which are relatively small and not all harmful^{9,11}.

Many discussions of the long-term effects of high densities on humans have failed to distinguish between the effects of density *per se* and the effects of other factors, such as poverty, which are commonly associated with high density. Winsborough¹², Schmitt¹³, and Galle *et al.*⁸ attempted to separate the effects of density from the effects of other socioeconomic factors by using partial correlation analysis. For example, they computed the partial correlation coefficient between density and mortality rate with income partialled out. This coefficient was interpreted as a measure of the association between density and mortality with income "held constant". Although this is a very common interpretation of partial correlation coefficients, it is an invalid one. This coefficient is only a measure of the amount of information which density gives about mortality above and beyond the information which income gives about mortality. Partial correlation analysis cannot be used to compare the relative effects of income and density *per se* on mortality. As discussed more fully by R. M. F.¹⁴, this common misinterpretation of partial correlation coefficients is based on a hidden circularity: one must make implicit assumptions about the presence or absence of causal relationships when in fact it is precisely these relationships which are to be discovered.

In addition there are serious inadequacies in the data used by Schmitt, Winsborough, and Galle *et al.* Schmitt¹³, in his

analysis of the correlates of density for Honolulu census tracts, did not adequately correct for the effects of age structure on his health measures, and used very crude measures of education and income as the controlled factors in his partial correlations. Winsborough¹² and Galle *et al.*⁸, in their analyses of the correlates of density for Chicago community areas, used the number of residents per square mile as a density measure. Some of the least dense areas by this measure were highly industrial or commercial, often with very high populations during business hours. In nearly half of the community areas, more than one-third of the land area was devoted to non-residential use. The number of persons per square mile was therefore a poor measure of residential density.

We present here two analyses of long-term effects of population density on human health. We control for the effects of other socioeconomic factors by comparing areas where these other factors do not differ, thus obviating the need for partial correlation analysis. Both analyses indicate that, within current ranges, population density, measured as the number of persons per area, does not have large effects on human health.

Correlates of Density in Chicago

Chicago has been divided into seventy-five internally homogeneous community areas, from which we selected ten, which in 1960 were similar with respect to eleven socioeconomic characteristics. (Data are for community areas 4, 15, 16, 17, 18, 19, 20, 25, 66 and 71 and are from ref. 15, except land areas, which are from ref. 16.) In each of these areas, less than 0.7% of the population was non-white or Puerto Rican; median family income was between \$7,306 and \$8,014; median years of school completed by persons over twenty-five varied from 9.6 to 11.2; 2.4-3.1% of the male labour force was unemployed and 35-52% were white collar workers; 45-55% were first or second generation immigrants to the United States; 56-67% of the females and 65-72% of the males aged fourteen and over were married; 23.5-30.5% of the population was less than eighteen years old; 1.4-4.0% of the housing was substandard; persons per household divided by median rooms per household was between 0.59 and 0.65; and between 8 and 25% of the land in each area was part of regions of 100 acres or more which were used for predominantly industrial and commercial purposes.

This sample of ten socioeconomically homogeneous community areas had densities ranging from 11,800 to 19,500 persons per square mile. There was also considerable variation in three related characteristics: proportion of housing units in structures with more than two dwelling units (6.5-68.5%); proportion of housing units occupied by their owners (28.0-80.5%); proportion of people living in the same quarters they occupied 5 years previously (39-51%). Community areas with higher densities had more multi-family dwellings, less home ownership, and greater geographical mobility (all rank-order correlations ≥ 0.9).

The rank-order correlation of density with age-standardized mortality is so low that it is more plausibly interpreted as due to sampling variation than as evidence of an actual relationship between density and health (Table 1). A graph of the data showed that there was no curvilinear relationship between density and mortality.

Juvenile delinquency and mental hospital admission rates had large and significant rank-order correlations with the

four related factors: high population density, high multiple dwelling units, high mobility, and low home ownership. We submit the following as the most plausible interpretations of these correlations: (1) higher density leads to increase in social pathology, specifically mental breakdown and juvenile delinquency; (2) home ownership leads to less pathology; (3) people with better physical and mental health have more success in becoming home owners; (4) pathology is increased by some aspect of multi-unit dwellings such as living above the ground floor or more disturbance from neighbours; (5) disruption of social relationships by more frequent changes in residence results in greater pathology; (6) less healthy and stable people move more often, so more mobility into an area means more pathology there; (7) people in high density regions are less tolerant of deviant behaviour and therefore more likely to turn over deviants to institutions such as mental hospitals or the police.

At present, we have no data on which to base a choice among these hypotheses, and indeed several of them may be valid simultaneously. The fourth hypothesis receives some support from Mitchell's observation that adults living above the ground floor in Hong Kong residences had higher hostility scores and more psychosomatic symptoms¹⁹. Some evidence in favour of the fifth hypothesis comes from previous studies in Chicago¹⁷ and Baltimore¹⁸. Lander's analyses showed that juvenile delinquency is highest in Baltimore census tracts that have the lowest owner-occupancy of dwellings and the most racial mixing (that is, intermediate proportions non-white). He interpreted this to mean that "anomie" or social disruption is an important cause of juvenile delinquency.

Although we are unable to choose among the seven hypotheses proposed above, we do feel that any other hypotheses we have been able to formulate are less probable than these are. For example, the pathologies at higher density cannot be attributed to more persons per room, since there was a slight tendency toward fewer persons per room at high density (presumably related to the slightly lower proportion of children). Similarly, the pathologies at higher density are probably not due to differences in education because higher juvenile delinquency, mental hospital admission, and mortality were correlated with more, not less, education in this sample of limited socioeconomic variability. None of the other socioeconomic factors was correlated with the three health measures.

If it should eventually be shown that the first of the seven hypotheses is correct and that density is a cause of juvenile delinquency and mental hospital admission, then it would be useful to have an estimate of the quantitative significance of density as compared to other causal factors. Unfortunately, as is discussed by R. M. F.¹⁴, this cannot be determined from the existing data since density is strongly correlated with the other factors.

In summary, as there is little or no correlation of mortality with density, it seems probable that density *per se* does not have a large effect on physical health. (Admittedly, we are not sure how long each person had experienced the density of a particular community area, for almost half the families

changed residences between 1955 and 1960, mostly within Chicago.) Higher density was correlated with higher mental hospital admissions and higher juvenile delinquency, but this correlation may well have been due to the fact that higher density was part of a syndrome including a higher proportion of buildings with more than two dwelling units, greater geographic mobility, and lower home ownership.

Density and Specific Diseases

In our second analysis of correlates of density, we have compared age-specific mortality rates for pairs of countries matched for *per capita* GNP and health care facilities. Obviously, the matching of socioeconomic factors is not nearly so precise in this second analysis as it was for the Chicago community areas. Also, the density measures conceal a good deal more heterogeneity. The differences in average density are, however, much larger, and the populations are large enough to allow us to make comparisons of mortality rates for specific causes and for the two sexes separately.

We chose pairs of countries for which one country was more than twice as dense as the other and also more highly urbanized²⁰, and for which both countries had similar literacy, income, and health facilities²¹ (Table 2). We used only countries with reliable vital statistics (as evaluated by the United Nations), more than 8,000 deaths annually for each sex, and less than 13% of deaths in the category "senility without mention of psychosis, ill-defined, and unknown causes", because this category tends to be used as a catch-all in poor statistical systems. For each country, we computed age-specific death rates by sex for total mortality, and for deaths due to twenty-two selected causes which were of special interest either because of their large magnitude or because of their relationship to the psychological condition of the individual. (Age-specific death rates were calculated from deaths²⁰ and population^{20,22} by 5-year age groups, except for England and Wales, by 10-year age groups.) The Wilcoxon matched-pairs signed-ranks test was used to compare age-specific death rates for total mortality and for each cause for each pair of countries. This non-parametric test evaluates the following null hypothesis: for two countries and for a particular cause-sex combination, the pairs of death rates for corresponding age groups are the same except for non-systematic deviations. The test emphasizes the consistency of the differences at different ages rather than the magnitude of such differences. Thus, rejection of the null hypothesis implies that the inhabitants of one country experience a higher mortality than the inhabitants of the other across much of the age-structure.

Table 2 displays the pairs of countries arranged in order of decreasing differences in density, and for each pair shows the density and socioeconomic measures, and the results of the comparisons of mortality for males and females using the Wilcoxon test. Out of the fourteen comparisons of total mortality, nine were not significantly different; in three comparisons, the high density country had higher mortality, whereas in two, the low density country had higher mortality.

Table 1 Correlations of Housing Variables with Health Measures in Chicago, 1960

	Density (persons per square mile)	Multiple dwellings (% of housing units in structures with more than two units)	Home ownership (% of housing units owner occupied)	Geographic mobility (% of persons over five who lived in a different house in 1955)
Male juvenile delinquents as a proportion of male population, aged 12-16	0.72	0.74	-0.69	0.77
Age-standardized rate of admissions to mental institutions in Illinois	0.63	0.78	-0.76	0.74
Age-standardized death rate	0.19	0.54	-0.50	0.44

Spearman rank order correlation coefficients between health and housing variables were calculated for ten socioeconomically similar community areas. Density and housing variables are highly intercorrelated. For a one-tailed test and $N=10$, 5% and 1% levels of confidence are attained, respectively, by coefficients of 0.56 and 0.75.

Malignant neoplasms, bronchitis, and ulcers were the three causes of death most consistently higher in the high density country. Tuberculosis, vascular lesions of the central nervous system, and accidents other than motor vehicle accidents were most consistently higher in the low density country. The causes listed in the middle of Table 2 showed no consistent relationship to density. (We have included in this middle group causes for which several consistent differences have been found, but in which France was the less dense country in almost all these cases. It was unfortunate that France was the low density country in three out of seven comparisons, but we were unable to find other pairs of countries that fit the specified criteria.) Note that homicide and suicide are among the causes of death which are not consistently related to density.

As in the first analysis, the chief conclusion seems to be that density does not have large effects on human health. The statistical tests were repeated for the younger and older age groups separately and revealed even fewer consistent differences than the tests for the whole population. Visual inspection of the graphs of age-specific death rates for each cause, sex, and pair of countries did not reveal any consistent differences which were not apparent from the statistical tests. We also computed age-adjusted death rates, which contain much less information than age-specific rates. In each of the 172 cases for which the Wilcoxon test showed a significant difference in the age-specific death rates, the age-adjusted rates differed in the same direction.

It is possible that some differences between high and low

Table 2 Comparisons of Age-specific, Cause-specific Death Rates for Countries with Similar Socioeconomic Conditions but Different Densities

Socioeconomic characteristic	Hong Kong/ Taiwan		England and Wales/ New Zealand		Netherlands/ Norway		West Germany/ Norway		England and Wales/ France		Netherlands/ France		West Germany/ France	
Density (persons km ⁻²)	3,770/360		320/10		375/12		233/12		320/91		375/91		233/91	
Population/1,000 acres of agricultural land	98,000/10,000		1,976/170		4,855/3,420		3,631/3,420		1,976/1,288		4,855/1,288		3,631/1,288	
% in cities with population more than 100,000 (city proper)	NA/32		41/17		32/23		30/23		41/19		32/19		30/19	
Gross domestic product <i>per capita</i> (US \$)	313/288		1,700/1,885		1,610/1,988		1,746/1,988		1,700/1,984		1,610/1,984		1,746/1,984	
Population per physician	2,320/2,330		870/830		860/790		630/790		870/850		860/850		630/850	
Population per hospital bed	280/1,040		100/100		130/110		90/110		100/100		130/100		90/100	
Cause of death for comparison of age-specific death rates	♂	♀	♂	♀	♂	♀	♂	♀	♂	♀	♂	♀	♂	♀
Total mortality		↓ ↓ ↓					↑ ↑ ↑	↑ ↑ ↑			↓ ↓ ↓			↑ ↑ ↑
Malignant neoplasms	↑ ↑ ↑	↑ ↑ ↑			↑ ↑	↑ ↑ ↑	↑ ↑ ↑	↑ ↑ ↑	↑			↑ ↑ ↑		↑ ↑ ↑
Bronchitis	↓ ↓ ↓	↓ ↓ ↓	↑ ↑		↑ ↑ ↑	↑ ↑	↑ ↑ ↑	↑ ↑ ↑	↑ ↑ ↑	↑ ↑ ↑	↑ ↑ ↑	↑ ↑ ↑	↑ ↑ ↑	↑ ↑ ↑
Ulcer of stomach and duodenum	↓ ↓ ↓	↓ ↓ ↓	↑ ↑ ↑	↑ ↑	↑ ↑ ↑		↑ ↑ ↑		↑ ↑ ↑	↑ ↑	↑ ↑ ↑	↑ ↑ ↑	↑ ↑ ↑	↑ ↑ ↑
Arteriosclerotic and degenerative heart disease	↑ ↑ ↑								↑ ↑ ↑	↑	↑ ↑ ↑	↑ ↑ ↑	↑ ↑ ↑	↑ ↑ ↑
Congenital malformations		↑							↑ ↑ ↑	↑ ↑ ↑	↑ ↑ ↑	↑ ↑ ↑	↑ ↑ ↑	↑
Hypertension with heart disease	↑				↓ ↓			↑ ↑ ↑	↑	↑	↑	↑	↑ ↑ ↑	↑ ↑ ↑
Motor vehicle accidents			↓ ↓ ↓		↑ ↑ ↑	↑ ↑ ↑	↑ ↑ ↑	↑ ↑ ↑					↑ ↑ ↑	↑ ↑ ↑
Pneumonia	↑ ↑ ↑		↑		↓ ↓ ↓	↓ ↓ ↓			↑ ↑ ↑	↑ ↑ ↑			↑ ↑ ↑	↑ ↑ ↑
Benign neoplasms					↑ ↑ ↑	↑ ↑ ↑	↑ ↑ ↑	↑ ↑ ↑	↓ ↓ ↓	↓ ↓ ↓				
Anaemias	↓ ↓	↓ ↓ ↓							↑ ↑ ↑	↑ ↑ ↑			↑ ↑ ↑	↑ ↑ ↑
Homicide							↑ ↑ ↑				↓ ↓ ↓		↑ ↑ ↑	↑ ↑ ↑
Diabetes mellitus				↓										↑ ↑ ↑
Syphilis and its sequelae			↑			↑ ↑ ↑		↑ ↑ ↑			↓	↓ ↓	↓	
Suicide	↓ ↓ ↓					↑ ↑ ↑	↑ ↑ ↑	↑ ↑ ↑	↓ ↓ ↓		↓ ↓ ↓	↓ ↓ ↓		↑ ↑ ↑
Nephritis and nephrosis	↓ ↓ ↓	↓ ↓ ↓				↑						↑		
Chronic rheumatic heart disease	↓			↑ ↑	↓	↓ ↓	↓ ↓ ↓	↓ ↓ ↓	↑ ↑ ↑	↑ ↑	↑ ↑ ↑	↑ ↑ ↑	↓ ↓ ↓	↓ ↓ ↓
Cirrhosis of liver	↓ ↓ ↓	↓ ↓ ↓	↓				↑ ↑ ↑	↑ ↑ ↑			↓ ↓ ↓	↓ ↓ ↓		
Other diseases of heart	↑ ↑		↓ ↓ ↓		↑ ↑ ↑	↑ ↑ ↑	↑ ↑ ↑	↑	↓ ↓ ↓	↓ ↓ ↓	↓ ↓ ↓	↓ ↓ ↓	↓ ↓ ↓	↓ ↓ ↓
Senility without psychosis and ill-defined and unknown causes					↓ ↓ ↓	↓ ↓ ↓			↓ ↓ ↓	↓ ↓ ↓	↓ ↓ ↓	↓ ↓ ↓	↓ ↓ ↓	↓ ↓ ↓
Tuberculosis		↓ ↓ ↓	↓		↓ ↓ ↓		↑ ↑ ↑	↑ ↑ ↑	↓ ↓ ↓	↓ ↓ ↓	↓ ↓ ↓	↓ ↓ ↓		↓
Vascular lesions affecting central nervous system		↓ ↓ ↓			↓ ↓ ↓	↓ ↓ ↓					↓ ↓			
Accidents other than motor vehicle	↓ ↓ ↓		↓		↓		↑ ↑		↓ ↓ ↓		↓ ↓ ↓	↓	↓ ↓ ↓	

For the mortality comparisons a group of arrows indicates that the age-specific death rates for this comparison are sufficiently different that the probability is less than or equal to 0.05, 0.02 or 0.01 (for one, two and three arrows respectively) that this difference could be due to sampling variations. Groups of arrows pointing up indicate that the denser country has higher death rates for that cause and groups of arrows pointing down indicate that the denser country has lower death rates for that cause. The data are for 1966 or 1967 with very few exceptions where it was necessary to use data from slightly earlier years.

density countries were concealed by methodological problems. Although we tried to equalize medical care, several of the high density countries may have better medical care: there are more hospital beds per capita in Hong Kong than in Taiwan; there may be more equitable access to medical care in the Netherlands and in England and Wales than in France. Such methodological problems would not, however, be sufficient to obscure the major differences in death rates which result from significant differences in education and income^{23,24}. Thus, at the very least we can conclude that the effects of density on health are much less than the effects of several other socioeconomic factors.

Higher air pollution in more dense, more urban countries may be a major cause of the higher death rates due to bronchitis and lung cancer²⁵ (the latter of which is a significant factor in the higher malignant neoplasm rates). It is obviously easier to attain low levels of air pollution in smaller, less dense aggregates of population, but current levels could be much reduced, even in high density regions, by better use of existing and foreseeable technology^{26,27}. Higher lung cancer is apparently also related to more migration²⁵ and more disruption of social groups⁷ in cities.

Implications

For ten socioeconomically similar community areas in Chicago, mortality is not correlated with population density. High mental hospital admission and juvenile delinquency are, however, associated with a syndrome of higher population density, more multiple-unit dwellings, less home ownership, and more mobility.

When countries matched for income and health care are compared, total mortality is not consistently related to population density. Cancer, bronchitis, and ulcer mortality are higher in denser, more urban countries, but deaths from other causes are lower.

Thus, in our two analyses, high population density is not strongly correlated with high mortality. It seems that, within current ranges, density *per se* is not a significant cause of poor physiological health. Our study has not revealed convincing evidence of effects of population density on mental health and crime, but our methods have not been adequate to exclude this possibility. Although it would be of interest to explore further the relationship of density to other variables, it seems clear that public policy to improve health should focus directly on more well-established problems such as poverty, malnutrition, inadequate education and health care.

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Prospects for Fusion Research in Britain

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More money is to be spent in Britain on fusion research in the next few years, but in the longer term international collaboration, for example within Europe, will become increasingly important.

It is now well known that matter may be converted into energy when atomic nuclei interact in such a way that their final mass is less than their mass at the start of the reaction.

Broadly speaking, the largest amounts of such nuclear energy are released either when the very heaviest nuclei in nature, such as uranium, divide into parts or when the very lightest, such as hydrogen, fuse together. The first of the two processes, the nuclear fission of heavy elements, is the basis of all the nuclear power stations at present working or under development. The second, the nuclear fusion of light elements, has so far been used to produce energy on a large scale only in thermonuclear explosions.

At the time of the development of the thermonuclear bomb, a search for a controlled method of releasing fusion energy was begun independently in the United States, the USSR, and Britain with the aim of finding a further large-

scale source of energy, to add to the opportunities already being created by the controlled release of fission energy. A fusion source of energy would have a virtually unlimited world supply of fuel represented by the relatively abundant heavy isotope of hydrogen, deuterium, present in, and easily separated from, seawater.

In order to achieve fusion reactions, temperatures of the order of hundreds of millions of degrees are required. As materials at these temperatures exist only as plasma, that is, a gas of positively charged atoms and negatively charged electrons, the chief emphasis to date has been on the search for a way of producing and then confining within a restricted space such plasmas for a long enough time to yield a useful net gain of energy. Because of the temperatures involved, a non-material method of isolating the plasma is required. So far, the most likely means of successfully confining plasma has seemed to be the use of magnetic fields and from the start the search has been devoted largely to studying the behaviour of plasmas in different configurations of such fields. Very recently, however, the possibilities of the inertial confinement of very dense plasmas generated from condensed fuel irradiated with a laser have attracted some attention—and this possibility is briefly discussed later in this article.

History

The easiest way to produce plasmas at high temperature seemed to be by using high current electrical discharges through low pressure gas, and hence research into the possibilities of producing thermonuclear conditions in such discharges was begun in Britain at three universities in about 1947. This continued until 1951 when the work was transferred to the Atomic Energy Research Establishment (AERE) Harwell and to AEI, Aldermaston Court. Work also began at the Atomic Weapons Research Establishment (AWRE) Aldermaston. About the same time, fusion work was also started in the United States. All this work was classified and guarded by security restrictions—as was most atomic energy research in those days.

The work remained classified for some years until, in a lecture at Harwell in 1956, Academician Kurchatov disclosed the results of research on pulsed high current discharges in deuterium, carried out in the USSR. Later that year, full cooperation on fusion research was agreed between Britain and the United States. This was followed in 1958 by declassification of fusion research in Britain, the United States and the USSR and the work of all countries was reported in detail at the Second Geneva Conference. Perhaps the most significant outcome of the conference was the wide appreciation that further progress depended in large measure upon a more fundamental understanding of plasma behaviour.

Nevertheless, the late 1950s were a time of great optimism for fusion and in 1958 when Zeta, the first substantial toroidal device, produced nuclear reactions at a rate “not inconsistent with thermonuclear production”, it was all too easy to conclude that success was at hand. Sadly, further work soon showed that this was a false dawn: the reactions were, it seems, produced not by a true temperature effect, but by a small percentage of the deuterons being accelerated by the intense electric fields generated inside Zeta by plasma instabilities. These plasma instabilities themselves resulted in a poor confinement, with confinement times of less than 100 μ s.

At about this time, the need to broaden the scientific base of fusion research led the United Kingdom Atomic Energy

Authority (UKAEA) to concentrate its fusion research into a new laboratory at Culham with the aim “to establish whether or not controlled fusion reactions in light elements can be achieved to provide a new source of nuclear power”. It was clearly recognized that the principal problem then facing fusion research was the difficulty of obtaining sufficient understanding to suppress plasma instabilities and thus to confine the plasma. The new Culham Laboratory was therefore oriented to work on this broad problem from the outset. The moves from Harwell and AWRE Aldermaston to Culham were completed in 1964. The work at AEI Aldermaston continued under a UKAEA contract until 1963, when it was stopped by the closure of this industrial laboratory.

The declassification of fusion research by Britain, the United States and the USSR in 1958 has given research on controlled thermonuclear reactions a strong and welcome international character. Thus, although this article deals primarily with the British programme, any broad appraisal of the fusion scene must take into account contributions from many countries—not only the ones in the field initially but also others, particularly France and Germany, each of whom have built up research programmes significantly larger than that in Britain.

By the mid-1960s, this global effort had revealed many aspects of plasma behaviour. It had been shown that one condition for the production of energy in practical amounts could be achieved: plasmas could be heated to the so-called “ignition temperature”, that is, the temperature at which the production of fusion power exceeds the energy losses by radiation. But a second essential condition, the confinement of plasmas for long enough times, remained unsolved and intractable.

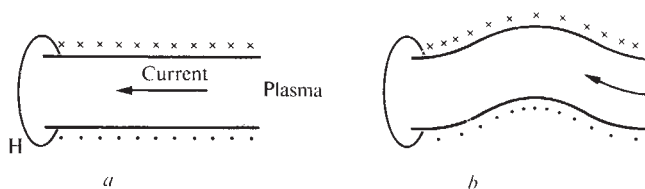


Fig. 1 Two situations in a Zeta-type apparatus. *a*, Magnetic field produces a pinching force towards the axis; *b*, a distortion in the magnetic field produces a net force which causes the distortion to grow.

The first threat to the confinement of high temperature plasma by magnetic fields came from the occurrence of “gross instabilities”. Theoretical analysis supported the experimental observation that many kinds of gross instabilities existed, all of which were sufficient to prevent the desired isolation of the plasma. This is not the place to give an exhaustive list of instabilities, but it is worthwhile illustrating the problem with just one example, namely the basic problem which faced a Zeta-type apparatus. In this type of device, the gas discharge itself produces an intense magnetic field and the hope is that this field will be sufficient to isolate the plasma. Thus, in an idealized quiescent situation, the situation can be imagined as shown in Fig. 1*a*, where the magnetic field produced by and surrounding the current may be shown to produce a force $\frac{1}{2} \mathbf{j} \times \mathbf{H}$ towards the axis, thus “pinching” the discharge into a smaller volume.

If, however, a distortion develops in the discharge, as shown in Fig. 1*b*, the magnetic field density becomes larger on one side of the discharge than on the other and this produces a net force which causes the distortion to grow rapidly.

In other words, the discharge is unstable to simple geometrical distortions of this type and in practice, unless additional magnetic fields are introduced, the kink in the discharge grows so large that the discharge hits the wall of the container.

In the mid-1960s, not only did gross instabilities, of which this is just one example, seem possibly too difficult to control but also, even if their control could be achieved (as it was in the stellerator in the United States), the ability of magnetic fields to confine plasma seemed to be further threatened by substantial energy losses associated with the diffusion of high temperature plasma across the confining fields at a much more rapid rate than classical diffusion theory indicated. This process, known as Bohm diffusion, had displayed itself in a number of experiments and had been found to be connected with the presence of density fluctuations in the plasma. It seemed that, even if a plasma could be created inside a magnetic field geometry which was stable overall, the plasma would nevertheless not be quiescent and the remaining fluctuations in the system would stimulate diffusion and loss of plasma.

By the mid-1960s, therefore, it seemed that the degree of magnetic confinement required to sustain a useful fusion reaction might be very difficult or even impossible of attainment because of the two problems of gross magnetic-hydrodynamic instability and Bohm diffusion. Indeed, appreciation of these difficulties could be said to have led to a period of gloom in fusion research throughout the world. It was not surprising therefore that, when the Culham programme was reviewed in 1966, the outcome was a decision by the UKAEA to decrease effort on fusion research progressively to half its initial level over a period of five years, the decision being subject to alteration if developments appeared to warrant it.

Recent Progress

The research programme at Culham has retained its high quality and has remained directed towards the confinement of plasmas and, in spite of the cutback, it has therefore been able to contribute significantly to the total world attack on this problem. This world effort has produced scientific progress which has been continuous and far reaching, so much so that gross instabilities have now been understood and suppressed in many containment systems and a number of remedies for Bohm diffusion have been devised. Indeed, classical or ideal plasma containment, which is actually better than that required for a fusion reactor, has already been achieved at "low" temperatures in several machines. These machines have, however, been designed for specific purposes and the twin objectives of adequate containment time and the achievement of ignition temperature have not yet been attained simultaneously in any one machine. This world effort has increasingly put emphasis upon toroidal systems as these allow the electrical discharge to close in upon itself—that is, eat its own tail—and allow magnetic field lines to be retained entirely within the confinement vessel itself.

It is interesting to note in passing how attitudes to Zeta, the first toroidal-pinch device, have fluctuated with the current understanding of magnetic containment. At one time it was easy for the non-expert to assume that the experiment was a disappointment. In fact, with increasing insight, the operation of Zeta in a stable mode was demonstrated and the device became a most valuable source of very hot plasma and, for some years, yielded many useful data pertinent to toroidal systems. Indeed, the experiment may be regarded as the natural precursor of the Tokamak

device, currently one of the most promising confinement systems.

Looking back over the past five years or so, from the British point of view, the chief milestones in scientific progress have been as follows: (1) the attainment of a detailed understanding of gross instabilities which led rapidly to their elimination by several schemes setting up magnetic fields of carefully defined geometry; (2) a growing understanding of Bohm diffusion and the demonstration of its suppression by several techniques; (3) the subsequent achievement of classical diffusion and prolonged confinement in suitably constructed devices, operating at "low" temperatures (that is, up to about a million degrees); (4) the development of laser scattering techniques which has transformed the detail and precision of experimental measurements; (5) the very successful Russian experiment on Tokamak which, together with the stabilization of the Zeta discharge, re-established toroidal discharges as credible confinement systems; (6) the evolution of sophisticated and comprehensive theoretical models to interpret and predict plasma behaviour in a wide variety of circumstances.

Present Programme

Currently, the British programme is based on four principal lines of study all aimed at resolving particular aspects of plasma containment in toroidal systems and bearing potential reactors in mind. It is unfortunately true that most experimental assemblies are difficult to describe in detail because the geometrical configuration of the magnetic field, which is set up to confine the plasma, is necessarily complex. Basically, however, in most toroidal systems the magnetic field lines follow the path that would be made by bending a helix into a circle so that the head and tail of the helix are joined up. An alternative way of picturing the magnetic field lines is to imagine a coil spring bent round into a circle to form a "doughnut" shape. The plasma is then trapped in the "doughnut-like" space inside the spring, that is, inside the magnetic field. The four systems in use at Culham set up this magnetic field in different ways. In the toroidal pinch assembly, which is the most direct successor to Zeta, the field is primarily set up by currents flowing in the discharge itself; this field is supplemented by an additional field produced by a coil wound on the toroid. The Tokamak geometry is closely related to this but with fields of different magnitude; thus the field generated by the discharge itself is dominated by the additional field produced by the external coil wound around the outside of the doughnut. In the superconducting levitron the field is produced partly by a coil wound round the outside of the doughnut and partly from a superconducting ring which is levitated within the doughnut space. In the stellerator the field is produced by a coil wound round the outside of the doughnut supplemented with a more complex winding, again on the outside of the doughnut, specially designed to produce the twisting effect analogous to a helix. These four systems are illustrated in Fig. 2a (the toroidal pinch and Tokamak), in Fig. 2b (the superconducting levitron) and Fig. 2c (the stellerator).

Supporting programmes are arranged to complement as effectively as possible all four chief approaches to confinement. Thus, studies of plasma production and heating are aimed at providing plasmas for experiments and exploring the techniques likely to be needed in reactors, whereas the technology programme is concerned principally with the development of superconducting coils for magnetic containment, surface physics, and the study of reactor concepts. Similarly, theoretical studies are related closely to the experimental programme with special emphasis on

plasma diffusion in closed line systems and on plasma turbulence. Diagnostic work has now been concentrated chiefly on the further development of laser scattering methods, while basic studies of plasma physics have been concentrated into one area, namely non-linear plasma effects.

In recent months a great deal of public attention has been given to the prospects of producing fusion reactions from the inertial confinement of very dense plasmas generated by lasers. Culham has had a modest programme of research on dense plasmas for some years, but very large programmes of work on this subject in the United States and the USSR have recently been reported although their work is only partly declassified at present. Because resources are limited, the Culham programme will be able to keep little more than a "watching brief" on this development but, because of the ingenuity and promise of the idea and because of its topical nature, it is worth a brief description here. The basic idea is very simple: the light from a powerful pulsed laser is focused by an optical system onto a small cold pellet of deuterium. When the laser pulse hits the outside of the pellet the outside layers of deuterium are evaporated off but the inner part of the pellet is compressed to a high density. Computer calculations suggest that compressions by as much as a factor of 10^4 can be achieved and this would be sufficient to produce a sizable release of thermonuclear energy. Furthermore, the highly compressed state of matter which a successful experiment would produce is of scientific interest in its own right.

There remain, however, a number of uncertainties connected with this idea. (1) It is not clear that sufficient laser energy can be deposited rapidly in the outer layers of the pellet. (2) The process of depositing this energy may cause fast electrons to run ahead of the incoming pressure pulse, thus building up a significant back pressure. (3) Unless the geometry is strictly spherical, electric currents may produce magnetic fields which would also stop the implosion. (4) It may not be possible to produce lasers of sufficient power.

Because of these uncertainties, the magnetic confinement of plasma still seems the most promising route to thermonuclear reactors. Nevertheless, modest compression ratios have already been produced by this laser technique, and substantial improvements are in sight once more powerful lasers are available and therefore this remains an exciting area of investigation which, at the very least, will need to be kept under close observation in the future.

Future Prospects

In view of the recent scientific progress, it is now possible to say with some confidence that plasma conditions close to those needed in a reactor could be achieved in a device based upon existing knowledge of magnetic containment but suitably scaled up in size. It is therefore probable that there is no fundamental bar to producing electric power from controlled thermonuclear fusion, though it is not possible at the present time to envisage clearly the form that a practical fusion reactor would take. What does seem quite clear is that the cost of pursuing a commercial fusion reactor (whatever designs are ultimately chosen) will at least equal that involved in introducing a major fission reactor system, namely some hundreds of millions of present-day pounds sterling. Such sums are very large indeed and therefore strong incentives exist for collaboration between different countries as a means, amongst other things, of effectively sharing the cost and risks of development.

It is not possible for any project, at the stage of development now reached with fusion research, to demonstrate that the major technological programme needed to exploit the ideas being developed will be economically worthwhile; not

enough can be known about the costs involved. In the case of fusion, it is impossible to assess the cost of producing electricity, either from a fusion reactor or from whatever may be the best alternative at the relevant time, with sufficient accuracy to show that the exploitation of the fusion reactor

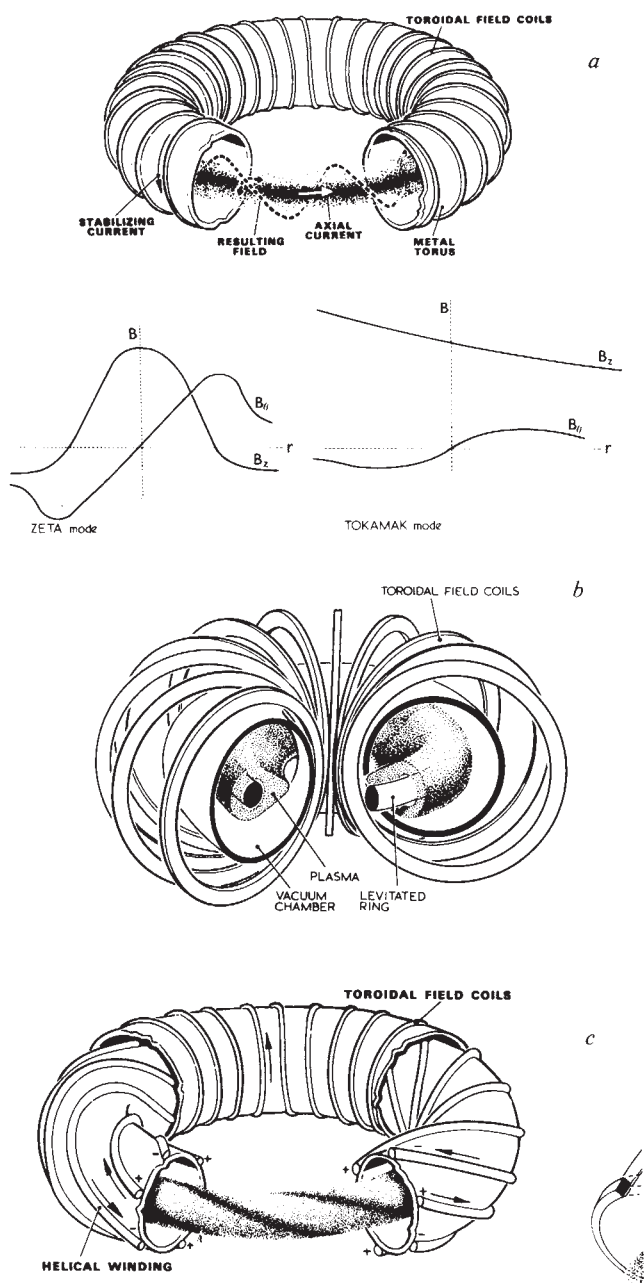


Fig. 2 Four fusion systems. *a*, Toroidal pinch and Tokamak; *b*, levitron; *c*, stellarator.

for electricity generation will save money. The best estimates that can be made now suggest that provided reasonable technological progress is made, for example, on the production of superconducting materials, the costs of electricity from fusion and from the best fission reactor will probably be about the same provided one uses today's prices for the uranium fuel needed to feed fission reactors.

This last qualification suggests that, if uranium were in short supply and prices rose, the fusion reaction might secure an economic advantage as well as offering a means of conserving world resources of energy. The world demand for electricity shows every sign of continuing to rise while reserves of fossil fuels become harder to find and more expensive to exploit. Indeed, most developed countries expect, in the light of these two trends, to have to generate some 30–70% of their total electric power requirements from nuclear fuels by the year 2000. If this nuclear component were to be generated solely by fission in thermal reactors then a uranium shortage could develop around the turn of the century and fusion reactors would fit in as natural successors to fission reactors on this broad time-scale. One actually expects, however, a substantial part of the world's nuclear generating capacity to consist by then of fast breeder reactors and these, by virtue of their highly efficient utilization of the fuel, could extend the life of low-cost uranium resources alone for at least a century and permit (with only a slight effect on the cost of power) the working of high cost reserves; this could defer the problem of energy sources for a very long time indeed. Indeed by extracting uranium from seawater—a feasible but expensive operation—the world's demands for energy could be met indefinitely by fast breeder reactors.

Even though no economic advantage from fusion reactors can now be proved, it should be noted that with the expected large increase in the demand for electricity, which can be met most economically by nuclear power, even a very small differential advantage for fusion could mean large savings arising from its exploitation. Moreover, although both fission and fusion reactors produce radioactivity, the amount of radioactivity is much less, and the potential problems are much easier to guard against, for fusion reactors than with any of the current fission reactors. This will give the fusion reactor environmental and consequently economic advantages.

Two contradictory conclusions could be drawn from this. First, that the economic advantages of fusion are uncertain and that the so-called "energy crisis" will be postponed for a very long time by the fast breeder reactor and that in consequence no work on controlled fusion should or need be done. Or, second, that it is now known that fusion reactors can be built, that with the immense effort now being devoted to them especially in the United States and the USSR, they almost certainly will be built (whether immediately economic or not) and that, although not enough is known to tell how much they will cost, they may possibly offer the option to enjoy cheaper and cleaner power than ever before.

Clearly difficult decisions will arise when the time comes for a major technological programme leading to commercial size fusion reactors. Work in Britain at present is, however, primarily scientific in character and modest in expenditure compared to that which eventually will be needed in a major "scaled up" programme. Looking to the future, it seems clear that a purely national research programme would be lost against the magnitude of the task. On the other hand, a European programme, if pursued in concert, would be likely to achieve technical success and could well produce commercial success at modest cost to each participating country. It would provide the best means of ensuring that Britain and Europe jointly and severally were able, when the time came, to occupy their proper place in the world market for fusion power.

Programme Review

The year 1972 marked the end of the rundown in the Culham programme decided upon five years previously by the UKAEA. To assist the authority in deciding what the

future programme should be, a panel of experienced scientific managers and administrators from industry, the Science Research Council, and universities, as well as from within the authority, under the chairmanship of Sir Harrie Massey, was asked to review the British fusion research programme and to make recommendations as to its broad future. The views of the panel were accepted by the authority, and the minister, Mr Boardman, has recently announced his acceptance of their recommendations which were broadly along the lines indicated in this article.

The minister made it clear that the government's decisions depended on full collaboration with Europe. Here, also, good progress is being made. The Euratom programme on fusion is very successful and Britain's accession to the EEC makes it natural for it to join in that programme as a full partner. The Council of Ministers of the community have indeed already agreed in principle that there should be a Contract of Association between the authority and the community in respect of the Culham fusion programme. The exact content of the future Culham programme will therefore be a matter for discussion with the appropriate Euratom boards. It is possible, however, to see the likely next steps in a Euratom programme. There is now a general feeling of confidence among fusion experts that plasma conditions approaching those needed in a reactor may be achieved in a device using what is already known about magnetic confinement but suitably scaled up in size. Such a machine with a capital cost of, say, £7 million would be considerably larger than those currently in use or under construction at Culham and would constitute the logical next step towards a fusion reactor. This logic has already been accepted by the United States, the USSR, and West Germany who propose to construct devices of this size and bring them into operation in the next three or four years. Each of these machines will, when built, represent a somewhat different approach to a reactor, and several more of similar size will be needed to explore this next stage. It seems likely that, when decisions are made within Euratom, Culham will be seen as a likely site for one of these larger machines and this will in its turn determine the main features of Culham's future programme.

The acceptance of the recommendations of the panel under Sir Harrie Massey, together with the prospective Contract of Association, imply a halt to the rundown of the fusion research programme at Culham and, indeed, over the next few years there will be a modest expansion of the work up to a point roughly halfway between the maximum size of the programme in 1966 and the minimum size reached in 1972. It seems fair to interpret this decision as a compliment, first to the scientific excellence of the world-wide work on fusion and second to the strenuous efforts made by Dr Pease and his colleagues at Culham to maintain a powerful and well-orientated effort in the face of a significant cutback of the programme over the past few years.

Increasing Involvement

Looking to the future, as fusion research gets closer to the point of commercial exploitation a European programme will need to involve industry more and more and it will be necessary to form international organizations involving governments, research and development authorities, industry and users. Such organizations are neither easy to form nor easy to manage. For the next few years, however, it seems sufficient to concentrate on welding together a good scientific team by building up on the existing and successful Euratom coordinated effort. The really difficult decisions on producing power from controlled fusion, which are very much larger and very much more complex than those that have to be made at present, will need to be faced in the future.

Possibility of Natural Diamond Synthesis under Conditions of Cavitation, occurring in a Fast-moving Magmatic Melt

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The formation of diamonds in nature can be explained by cavitation occurring in magma flowing in kimberlite pipes.

THE greatest difficulty in understanding the origin of natural diamonds is to discover a geological situation in which pressures above 70 to 100 kbar and temperatures above 1,200° C are reached. In some cases diamonds have been seed grown under low pressure ($P=1$ torr). But this method is feasible only when building up a crystal lattice, and not when synthesizing it anew¹. Moreover, a marked fractionation of carbon isotopes² accompanying the formation of such diamonds would result in an isotopic composition of diamonds which is quite untypical for natural conditions. The mineralogical composition of the diamond bearing rock kimberlite indicates that it was never subjected to pressures above 20 to 25 kbar: it contains pyrope (synthesis pressure 10 to 20 kbar), but not minerals formed under pressures of 35 to 90 kbar such as coecite, stishovite (dense modifications of SiO_2), and piezolite (artificial aluminosilicate, synthesized under $P=45$ kbar and $T=700^\circ\text{C}$), whose formation must precede that of diamonds. Here I draw attention to the existence of a hydrodynamic phenomenon, cavitation, in which high pressures and temperatures occur at several points, whereas the ambient parameters are, on average, moderate. Cavitation occurs in fluids containing dissolved gases, and high gas contents are distinctive features of diamond-bearing kimberlite magma, saturated with carbon dioxide, whose relics invariably occur in kimberlites as dispersed carbonates.

Under a certain initial pressure P_0 the flow is a single phase fluid-gas. As the pressure drops below a critical value, perhaps as a result of increased flow rate, incipient bubbles of gas come into existence. Now, if the pressure rises again before the gas bubbles have associated into a continuous phase, the bubbles will collapse. In these conditions the pressures at the points towards which the bubbles collapse are greater, by several orders, than the initial ambient pressure. Simultaneously, because of adiabatic compression, the temperature will increase. Such cavitation is known to cause erosion and destruction of propeller blades, as well as components of pumps and pipings.

Kimberlite pipes (diatremes) typically extend vertically with a length of the order of several kilometres and diameter of the order of tens of metres. Under such circumstances cavitation inception is associated with magma flowing quickly in a channel of variable cross section.

According to Bernoulli's relation, pressure across contracted portions of a channel will decrease as

$$P_0 - P = \frac{\rho}{2} \bar{u}^2 \left[\left(\frac{D}{d} \right)^4 - 1 \right] \quad (1)$$

where D = mean diameter of channel; d = diameter of contraction; $\bar{u}$ = average rate of magma through channel.

The initial pressure P_0 in the magma should meet the requirements of synthesis for the most barophilous minerals of kimberlite rocks, whose formation excludes the cavitation synthesis mechanism; these include pyrope. Because pyrope is synthesized at pressures approaching 20 kbar, this value should be regarded as the required original pressure.

The average velocity u is controlled by hydraulic losses in the magmatic channel; with $D = n \times 10$ m, u is about $n \times 100$ m s^{-1} . The pressure under which carbon dioxide fully dissolves in magma at approximately 1,000° C is ~ 11 kbar. Thus, it will be necessary to reduce the channel diameter no more than 2 to 3 times to produce the pressure required to cause cavitation in a magmatic melt.

The question whether the high pressures and temperatures necessary for the synthesis of diamonds do occur in the process of gas bubble collapse can be answered by calculating the collapsing conditions.

Introducing simplifying assumptions (ideal character of compressed gas, negligence of dissipative processes, and compressibility and viscosity of fluid) the process of gas bubble collapse can be expressed as follows³:

$$\left(\frac{dR}{dt} \right)^2 = \frac{2P}{3\rho} \left[\left(\frac{R_0}{R} \right)^3 - 1 \right] - \frac{Q \left[\left(\frac{R_0}{R} \right)^3 - \left(\frac{R_0}{R} \right)^{3\gamma} \right]}{1 - \gamma} \quad (2)$$

where P = external pressure (which can be assumed unchanged and equal to P_0); ρ = fluid density; R_0 = maximum radius of bubble at the initial moment of collapse; Q = gas pressure in bubble at $R = R_0$; γ = adiabatic index.

The maximum pressure in the matter being compressed by the closing walls of a bubble can be deduced from the relation

$$P_{\max} = Q(R_0/R_{\min})^{3\gamma} \quad (3)$$

where $R_{\min}$ = minimum size of a bubble reached during collapse.

The maximum size of a bubble is dependent on the magnitude of rarefaction ΔP at the moment of gas bubble growth, the fluid density ρ and the bubble growth time t_g , and is defined by the relation

$$R_0 = (2\Delta P/3\rho)^{1/2} t_g \quad (4)$$

The time of growth can be defined as the period during which the fluid passes the channel contraction zone.

An approximate calculation shows that at the moment a gas bubble collapses in a medium with original pressure near 20 kbar ($R_0 = 20$ cm, $R_{\min} = 0.5$ cm), the matter is compressed at a pressure of n 1,000 kbar, substantially greater than that obtainable artificially or conceivable in any other natural processes. The matter of a gas-filled bubble, when quickly compressed, can be adiabatically heated to temperatures much greater than the initial T_0 :

$$T_{\max} = T_0(R_0/R_{\min})^{3\gamma-3} \quad (5)$$

The adiabatic increase of temperature in the points of bubble collapse removes the necessity of a high initial temperature of the ambient medium and permits a T_0 of 1,000–1,100° C at

which a newly formed diamond, after dissipation of the pressure in the zone of compression, will be in conditions corresponding to metastability. The temperature of magma has been considered to be at least 1,500° C on the basis of the PT conditions of diamond synthesis. But in the absence of a special medium⁴, this temperature should be at least 2,000 K. Apart from the difficulties in accounting for a transition of diamond into a metastable state, this is inconsistent with the presence of minerals associated with the diamond and having a relatively low melting temperature; also contact phenomena are poorly expressed (absence of fused walls in the magmatic channel and absence of xenolites—debris of intrusive rocks, carried by magma).

There is also the lower temperature boundary, limited by magma viscosity. Investigation of the collapse of a spherical space, taking due account of the fluid viscosity⁵, shows that the dynamics of the process in this case depends on the Reynolds number:

$$R_e = \frac{R_0}{\nu} \sqrt{\frac{P_0}{\rho}} \quad (6)$$

where ν = kinematic viscosity. Viscosity impedes fluid acceleration during collapsing. At $T = 800^\circ \text{C}$ the viscosity has such a pronounced effect that for plausible initial radii the Reynolds numbers fall below the critical values (the critical value of the Reynolds number $R_{e1} = 8.4$).

Thus, the optimum temperature of a melt clearly approaches 1,000 to 1,100° C. It is just at this initial temperature and at the original pressure, $P \approx 20$ kbar, that the conditions occurring in the zone of compression of cavitation bubbles conform to a diamond-stable domain.

This does not solve the entire problem of synthesis, because the kinetics and relaxation conditions are also important. The process of diamond synthesis does proceed sufficiently quickly. This is proved by the production of synthetic diamonds by explosion, in which high pressures (300 to 500 kbar) are maintained for not longer than $(6-10) \times 10^{-6}$ s (ref. 6). The time of bubble collapse is found by solving equation (1), which at $\gamma = 4/3$ gives the relation

$$t_{\text{coll}} = R_{\min} \sqrt{\frac{2\rho(\xi-1)}{P_{\max}}} \left[1 + \frac{2}{3}(\xi-1) + \frac{1}{5}(\xi-1)^2 \right] \quad (7)$$

where $\xi = R_0/R_{\min}$. Substituting numerical quantities of appropriate values, $t_{\text{coll}} = 2 \times 10^{-3}$ s. This exceeds by almost three orders of magnitude the time required for diamond synthesis in explosions.

In addition the rate of bubble wall convergence may, over a certain element of convergency radius, exceed the speed of sound in the medium. As a result, a spherically converging shock wave may break off the bubble surface. The occurrence of high pressures in the vicinity of the shock wave focus must result in the inception of a diamond seed at the centre of the bubble. The lattice of this diamond seed will govern the crystallization of the main body of the diamond under the compressing effects of the bubble converging walls.

The problem of relaxation lies in the necessity to provide a very rapid transition of synthesized diamonds into a metastable state. After the pressure drops to 1 atm, the diamond remains stable at temperatures less than 1,000° C; at a pressure of 20 kbar the diamond can remain stable up to 2,000° C (ref. 7).

If temperatures remain above these limits after the pressures reduce, the diamond will leave the boundaries of stability and transform into graphite. Dissipation of heat, following the synthesis process, is always slower than the pressure decrease. Therefore, graphitization seems to be inevitable. But even assuming that diamond somehow cools down rather quickly, mechanical stresses, arising in a crystal because of a temperature gradient, will result in cracking and destruction of the diamond, this being the quicker the larger is the crystal. Nevertheless, large and perfect crystals do occur naturally.

This can be explained by examining the cavitation effect. At superhigh pressures, built up during the collapse of a gas bubble, the diamond compression is found to be sufficient for thermodynamic self-cooling to take place as a result of adiabatic expansion which accompanies the super high pressures. Adiabatic cooling is possible only when an expanding body does certain work against pressure forces, acting from outside. This condition is not satisfied, for example, in technical devices of static compression, in which the pressure is released from outside; but it is brought about during cavitation synthesis, because here the process of relaxation is accompanied by the inception of an inverse shock wave, which is formed because of the resilient expansion of compressed matter.

The temperature decrease can easily be evaluated. Entropy variation as a total differential at parameters T and P is given by

$$dS = \left(\frac{\partial S}{\partial T} \right)_P dT + \left(\frac{\partial S}{\partial P} \right)_T dP$$

From the adiabatic process relation, $dS = 0$. Taking into account the well-known correlations of thermodynamics,

$$\left(\frac{\partial S}{\partial T} \right)_P = \frac{C_P}{T} \text{ and } \left(\frac{\partial S}{\partial P} \right)_T = - \left(\frac{\partial V}{\partial T} \right)_P$$

and bearing in mind that $\frac{1}{V} \left(\frac{\partial V}{\partial T} \right)_P = \alpha$

where α = thermal expansion coefficient,

$$(C_P/T) dT = \alpha V dP$$

Integrating and assuming C_P is independent of temperature

$$\ln \frac{T_{\max}}{T_0} = \frac{\alpha T_0 V}{C_{P_{\max}}} (P_{\max} - P_0) \quad (8)$$

Assuming $\alpha = n \times 10^{-5} \text{ deg}^{-1}$, $V = 3.43 \text{ cm}^3 \text{ mol}^{-1}$, $C_P = 6 \text{ kcalorie deg}^{-1} \text{ mol}^{-1}$

$$\ln \frac{T_{\max}}{T_0} \sim 10^{-6} P_{\max} \quad (9)$$

Thus, at pressures $P_{\max} = 100$ kbar the adiabatic decrease of temperature is only a few degrees, negligibly small for the ultimate pressures in technical devices, as well as for the ultimate pressures in any conceivable geotectonic process. But, as shown earlier, pressures in excess of 1,000 kbar are encountered in cavitation. In this case the temperature may decrease adiabatically 3 to 10 or more times. Thus, at the peak pressure and synthesis, 2,000 kbar, the temperature in the relaxation process will decrease by 1,000 K, even if the maximum temperature is 10,000 K.

Two unique situations are at once created. First, the diamond momentarily attains a metastable state—it passes the entire process of relaxation in the domain of its thermodynamic stability, completely avoiding graphitization. Second, because of a momentary decrease in temperature, no mechanical stresses occur because cooling is effected through losses of internal energy and not by heat transfer.

Many problems concerning the origin of diamonds can thus be explained in terms of cavitation.

The suggested mechanism of diamond synthesis not only shows how the required pressures and temperatures occur under natural conditions, but also explains why diamonds are found imbedded into rocks which are free of any traces of those very high pressures and temperatures necessary for diamond formation.

Because in the course of cavitation synthesis the inception of each crystal proceeds individually, there are no limitations involving the total concentration of carbon in the medium. These limitations arise from the idea that diamonds crystallize out of a supersaturated solution, this being feasible only at a sufficiently high concentration of carbon in a melt ($\approx 30\%$).

ref. 8). That has been one of the most difficult problems of diamond origin because carbon never occurs naturally in such high concentrations.

Many natural diamonds have peculiar internal morphologies indicative of a series of interruptions during their synthesis. This is in accordance with the pulsatory movement of gas bubbles caused by compression⁹. Moreover the newly formed diamond surrounded by a gaseous mantle is the most likely nucleus for cavitation when suitable conditions arise again.

These ideas explain the occurrence of neighbouring empty and diamond-bearing pipes; the crystallographic specificity of diamonds in individual pipes; the presence of diamonds in kimberlite rock as individual crystals; the occurrence of diamonds exactly in kimberlite, which is a unique kind of CO₂-saturated fast moving magma; the similar carbon isotopic composition of diamond and endogenetic carbon dioxide; the paragenesis with pyrope and diamond; and the phenomenon of the split of diamonds and separate grains of other minerals while bodies such as xenolites remain untouched.

Finally, why have large crystallographically perfect diamonds, such as those occurring naturally, never been made artificially, in spite of the thorough selection of reactive systems, accurate control of the process and application of amply high temperatures and pressures? Evidently, because the cavitation technique used *in vivo* has never yet been tried in the laboratory.

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The Pliocene Time Scale: Calibration of Planktonic Foraminiferal and Calcareous Nannoplankton Zones

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The Pliocene spans the interval between 5 and 1.8 m.y.; its lower and upper limits can be determined by multiple micropalaeontological criteria. Within the Pliocene twelve planktonic foraminiferal and seven calcareous nannoplankton datum levels can be calibrated to the palaeomagnetic time scale.

ESTIMATES on the time span of the Pliocene have varied widely, from a little over 1 m.y.^{1–5} to over 10 m.y.^{6,7}. The age of the Miocene/Pliocene boundary itself has been estimated to be as young as 2.7–3.0 m.y.^{1–4} and as old as 15 m.y.⁸. Estimates by most vertebrate palaeontologists have ranged between 10–12 m.y. because of the supposed initial appearance of the three-toed *Hipparion* in the lower part of the stratotype Pontian of the eastern Mediterranean, which was thought to coincide with the Miocene/Pliocene boundary^{9,10}. More recent estimates of 7 m.y. for the Mt Capanne granodiorite on Elba¹¹, and 6.6–8.0 m.y. for the volcanic tuffs intercalated with Messinian beds in Morocco¹², have indicated that the Miocene/Pliocene boundary, as defined in the marine sedimentary succession in the Mediterranean region, is younger than 7 m.y. The date of 4.7 m.y. on the Orciatico trachyte which metamorphoses lower Pliocene clays in Tuscany¹³ and integrated biostratigraphic palaeomagnetic studies on deep-sea cores¹⁴ have indicated that the boundary is older than 4.5 m.y. and an age of 5 m.y. has been suggested^{15,16}. Recent investigations have corroborated this estimate^{17–19}.

Similarly, age estimates of the Pliocene/Pleistocene boundary

have varied over a wide range from about 0.6 m.y.^{20,21} (Emiliani qualified these estimates with a caution that correlation with the base of the stratotype Calabrian in Italy had not been achieved) to over 4 m.y. based on the dates of 3.4 (ref. 22) to 4.3 (ref. 23) m.y. on the earliest Villafranchian faunas which are attributed by many vertebrate palaeontologists in Europe and the Soviet Union to the Pleistocene. Recent data have shown that the continental type Villafranchian is essentially late Pliocene (pre-Calabrian), that the Villafranchian in a wide sense is as young as 1.0 m.y.²⁴, and that the base of the stratotype Calabrian at Santa Maria di Catanzaro, that is, the Pliocene/Pleistocene boundary, is about 1.8 m.y. old^{25–27}.

I present here an estimate on the age of the Miocene/Pliocene boundary based on considerably firmer data than those available up to now^{28–32}, suggest a new planktonic foraminiferal zonation of the Pliocene, correlate these zones with the Pliocene calcareous nannofossil zones and calibrate them both to the palaeomagnetic time scale.

Age of the Miocene/Pliocene Boundary

The development of a discrete supplementary aperture on the spiral side of the test has been suggested as the distinguishing criterion separating the planktonic foraminiferal genus *Sphaeroidinella* from its ancestor *Sphaeroidinellopsis*³³. The initial evolutionary appearance of *Sphaeroidinella dehiscentis* (including *forma immatura*) from its immediate ancestor *Sphaeroidinellopsis subdehiscentis paenedehiscentis* was said to denote the base of Zone N19 (ref. 34). The initial appearance of *S. dehiscentis* was recorded about 40 feet above the base of the Zanclean stage in Sicily³⁵ so that the Miocene/Pliocene boundary was said to lie within the subjacent Zone N18. The base of Zone N18 is placed at the bottom of the first evolutionary occurrence of *Globorotalia tumida* from its immediate ancestor, *G. plesiotumida*³⁶.

Table 1 Placement of Miocene/Pliocene Boundary by Calcareous Nannoplankton Biostratigraphers

PALEO- MAG. TIME SCALE	T in my	EPOCH SERIES		AGE STAGE	C A L C A R E O U S N A N N O P L A N K T O N Z O N A T I O N				MIOCENE				
					MARTINI WORSLEY(1970)		BUKRY (1971)		PLIOCENE BOUNDARY				
					ZONE		SUBZONE						
G I L B E R T NUN. COCH.	3.6	P L I O C E N E	E A R L Y	Z A N C L I A N	Reticulofenestra pseudumbilica	NN15	Ceratolithus tricorniculatus	Ceratolithus rugosus	← BUKRY (1971,1972) MARTINI & WORSLEY (1970) MARTINI (1971)				
	4.0				Discoaster asymmetricus	NN14				Ceratolithus amplificus	← BUKRY(1972), GARTNER (1973)		
	4.4				Ceratolithus rugosus	NN13						Ceratolithus tricorniculatus	Ceratolithus rugosus
	4.8				Ceratolithus	NN12							
5.2	M I O C E N E	L A T E	M E S S I N I A N	iricorniculatus	Triquetrorhab- dulus rugosus		← BUKRY (1971,1973) GARTNER (1969)						
5.6													
6.0													
6.4													
EPOCH 6	6.4	M I O C E N E	L A T E	M E S S I N I A N	Discoaster quinqueramus	NN11			← BUKRY (1973)				
EPOCH 7	6.8												
7.2													

The *S. dehiscens* Datum, as originally defined by Bandy^{37,38}, refers to the initial appearance of large, robust individuals of *S. dehiscens* with a broad, scalloped, everted flange. This level is associated with the extinction of the *Sphaeroidinellopsis* group, is slightly older than the extinction level of *G. multicamerata* and *Globoquadrina altispira*, is coincidental with the planktonic foraminiferal N20/N21 zonal boundary and has been shown³⁹ to be about 3 m.y. old on the palaeomagnetic time scale. It does not correspond to the Miocene/Pliocene boundary as defined in the marine sedimentary succession in the Mediterranean.

Thus we see that the definition of the *S. dehiscens* Datum by both Bandy^{37,38} and Blow³⁴ is clearly and unequivocally stated but refers to biostratigraphic events which are significantly different in space and time. The *S. dehiscens* Datum of Bandy^{37,38} is about 3 m.y. old¹⁴; that of Blow³⁴ about 4.8 m.y. old¹⁹ (see also Tables 2, 3 and Fig. 1).

In terms of the calcareous nannofossils the Miocene/Pliocene

boundary has been variously placed at the base of the *Ceratolithus tricorniculatus* Zone (NN12)⁴⁰, within the *C. tricorniculatus* Zone⁴¹⁻⁴³, at the *C. tricorniculatus*/*C. rugosus* (NN12/NN13) boundary^{44,45}, or within the *C. rugosus* Zone (NN13)⁴⁶⁻⁴⁹ (Table 1). In a recent investigation⁵⁰ the Miocene/Pliocene boundary is correlated with the *C. rugosus* Datum (base Zone NN13). This was based on an earlier study⁵¹ showing that the *C. rugosus* Datum occurred in the southwest Pacific core CAP 38 BP at a level (625 cm) dated within Zone N18 on planktonic foraminifera⁵².

S. dehiscens has been shown¹⁴ to range well below the age of 4.2 and 4.1 m.y. estimated for the two levels determined by Parker⁵² and Blow⁵³, respectively, in core LSDH 78 P for the

Table 3 Estimated Age of Biostratigraphically Important Pliocene Datum Levels in Core 6, Hole IIIA (Orphan Knoll)

Sec-	Depth	Estim-	Calcareous	Planktonic foraminiferal
tion	(cm)	ated age	nannoplankton	datum
		(m.y.)	datum	
2	134	2.9	—	<i>Globoquadrina altispira</i> and <i>Sphaeroidinellopsis</i> spp. (E)
	140	3.05	<i>Reticulofenestra pseudumbilica</i> (E)	—
3	10	3.48	<i>Ceratolithus tricorniculatus</i> (E)	—
	18	3.7	—	<i>Globigerina nepenthes</i> (E)
	55	4.45	<i>Ceratolithus rugosus</i> (FO)	—
	70	4.8	—	<i>Sphaeroidinella dehiscens</i> (FO)
	75	4.9	—	<i>Globoquadrina tumida</i> (FO)
	80	5.0	<i>Ceratolithus amplificus</i> (FO)	<i>Globoquadrina dehiscens</i> (E)

Table 2 Pliocene Planktonic Foraminiferal Datum Levels

Time (m.y.)	Datum level	First occurrence (FO)	Last occurrence (LO)
~1.8	III <i>Globigerinoides fistulosus</i>	(Top Olduvai)	LO
~1.95	IV <i>Globoquadrina truncatulinoides</i>	(Base Olduvai)	FO
~2.95	V <i>Sphaeroidinellopsis</i> spp.		LO
~3.34	VI <i>Globoquadrina margaritae</i>		LO
~3.6	VII <i>Pulleniatina</i> (primarily <i>P. primalis</i>)	Sinistral to dextral coiling change	LO
~3.7	VIII <i>Globigerina nepenthes</i>		FO
~4.3	IX <i>Pulleniatina spectabilis</i>		FO
~4.8	X <i>Sphaeroidinella dehiscens</i>		FO
~4.9	XI <i>Globoquadrina tumida</i>		FO
~5.0	XII <i>Globoquadrina dehiscens</i>		LO

Data from refs. 14, 19 and this paper.

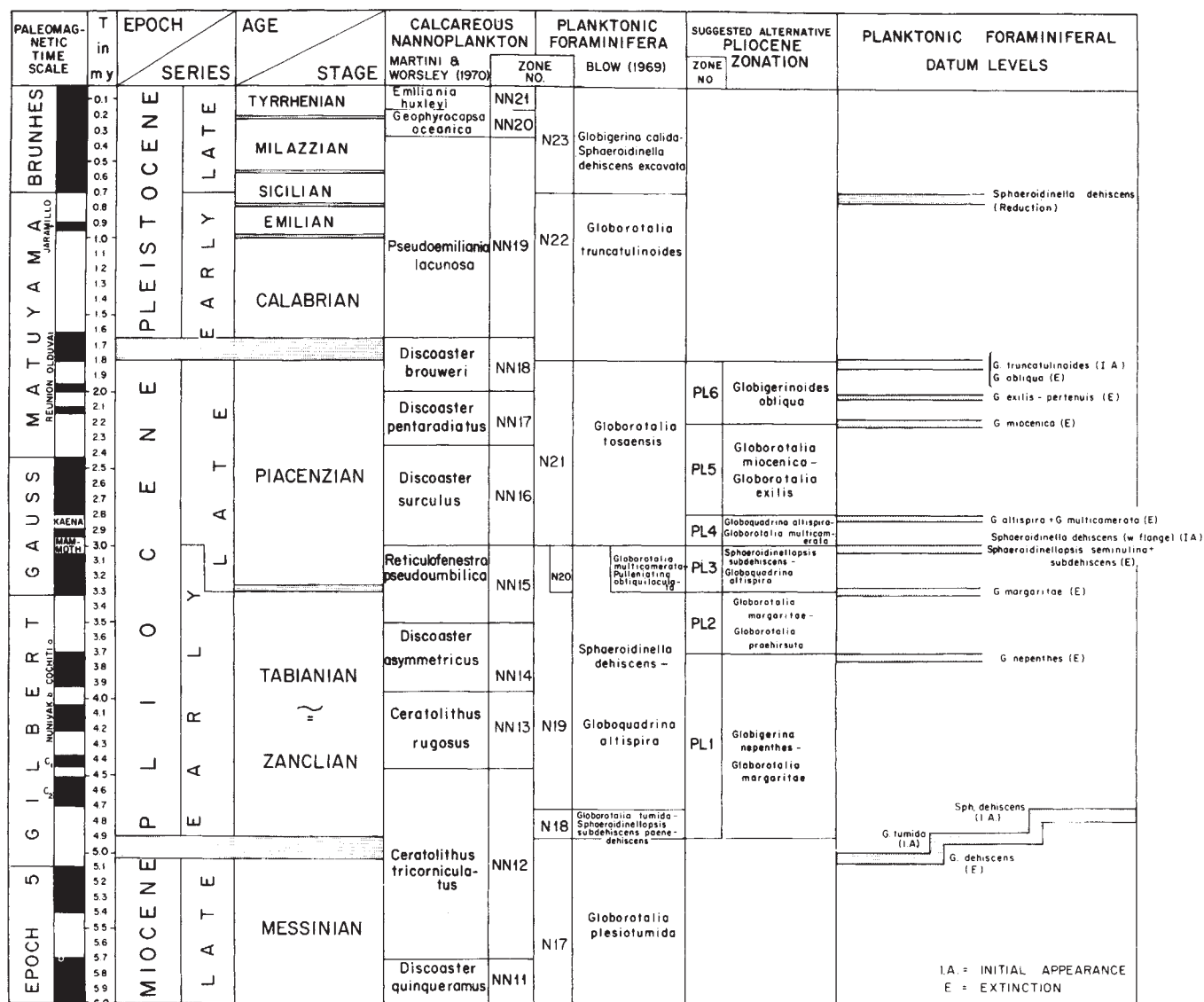


Fig. 1 Correlation and calibration of Pliocene planktonic foraminiferal and calcareous nannoplankton zones and datum levels.

N18/N19 boundary and of 4.3 m.y. for the base of Zone N18 (=Miocene/Pliocene boundary) by extrapolation in that core¹⁴. In fact it was shown¹⁴ that the Miocene/Pliocene boundary must be older than 4.5 m.y., the age of the bottom of core V24-59 in which *S. dehiscent* was present. The initial appearance of *C. rugosus* is recorded within the Gilbert "C" in cores V24-59 and RC12-66 at about 4.45 m.y.⁵⁰. Thus, the Miocene/Pliocene boundary, as determined by planktonic foraminiferal criteria on core LSDH 78 P (refs. 52, 53) and on CAP 38 BP (ref. 52), is younger than the true Miocene/Pliocene boundary using the criteria originally suggested for recognizing this boundary. The *S. dehiscent* Datum (base Zone N19) and the *G. tumida* Datum (base Zone N18) are dated in this paper at 4.8 and 4.9 m.y. respectively and this is in perfect agreement with independent estimates on these levels¹⁹. I conclude that the estimate of the Miocene/Pliocene boundary at the base of the *C. rugosus* Zone (~4.5 m.y.)⁵⁰ is too young and that this level lies within the lower Pliocene.

It should be pointed out that the recognition of the *S. dehiscent* Datum can be extremely difficult; thus, if the first appearance of *S. dehiscent* is not accurately determined miscorrelation by as much as a whole nannofossil zone may result. For this reason the first appearance of *G. tumida* and the extinction of *Globoquadrina dehiscent* (~5.0 m.y.) are here considered more reliable indicators of the Miocene/Pliocene

boundary. The last occurrence of *Globoquadrina dehiscent* was recorded at 674-676 cm in CAP 38 BP (ref. 52), a little over 0.5 m below the initial appearance of *C. rugosus* at 625 cm (ref. 51).

The Miocene/Pliocene boundary has been correlated with the first appearance of *C. amplifucus*⁴², a species intermediate between *C. tricorniculatus* and *C. rugosus*. *C. amplifucus* appears at a level (18 m) dated at about 4.9 m.y. in core RC12-66 (ref. 19) and is coincidental with the base of Zone N18 in that core.

The co-occurrence of *C. tricorniculatus* and *C. amplifucus* is restricted to the lower part of the lower Pliocene (Zancian) in Sicily⁵⁰. *C. amplifucus* initially appears approximately 6 m (but does not become abundant until about 18 m) above the base of the Zancian (*S. Gartner*, personal communication) at the newly proposed type section at Capo Rosello⁵⁴. If this level corresponds to the level dated at about 4.9 m.y. in core RC12-66, which is approximately equivalent to the base of Zone N18, then the Miocene/Pliocene boundary may be somewhat older than this level and an estimate of 5.0 m.y. is suggested here. The initial appearance of *S. dehiscent* was recorded³⁵ about 40 feet above the base of the Zancian so that the Miocene/Pliocene boundary is somewhat older than the N18/N19 boundary. As noted below, it actually lies at the base of Zone N18 or even slightly lower; for all practical

purposes it may be drawn at the base of Zone N18. Thus the Miocene/Pliocene boundary lies within the stratigraphic range of *C. tricorniculatus* (NN12) and in a recent subdivision of the *C. tricorniculatus* Zone into three subzones (from the bottom), *Triquetrorhabdulus rugosus*, *C. amplificus* and *C. rugosus*, the Miocene/Pliocene boundary was drawn at the base of the *C. amplificus* Subzone⁴².

In summary, it seems that a set of multiple criteria have been developed for determining the Miocene/Pliocene boundary in a way similar to that suggested for the Pliocene/Pleistocene boundary⁵⁵. These criteria include: planktonic foraminifera—extinction of *Globoquadrina dehiscens*, first evolutionary appearance of *S. dehiscens* and *G. tumida*; calcareous nannoplankton—first evolutionary appearance of *C. amplificus*. These multiple criteria occur within the lower part of the Gilbert Reversed Epoch on the palaeomagnetic time scale. The Gilbert/Epoch 5 boundary has been dated at about 5.2 m.y. (A date of 5.1 m.y. was estimated for the top of Magnetic Epoch 5 by Foster and Opdyke⁵⁶ and revised by Talwani *et al.*⁵⁷ to 5.18 m.y.). For practical purposes the Miocene/Pliocene boundary may be said to occur within the interval of 4.9–5.1 m.y.

Correlation and Calibration of Pliocene Calcareous Planktonic Zones

The basic parameters within which the Pliocene events are calibrated are the Pliocene/Pleistocene boundary (~1.8 m.y.) and the Miocene/Pliocene boundary (~5.0 m.y.). The former is characterized by several biostratigraphical events which, taken together, approximate the upper boundary of the Pliocene²⁷. They include: extinction of *Globigerinoides obliqua extrema* at the base of the Olduvai Event, about 1.8 m.y.; initial appearance of *G. truncatulinoides* somewhat higher, within the lower part of the Olduvai Event, about 1.75 m.y.; extinction of discoasters near the top of the Olduvai Event, about 1.65 m.y.; extinction of *Globigerinoides fistulosa* slightly above the top of the Olduvai, about 1.60 m.y. The Pliocene/Pleistocene boundary is drawn here, arbitrarily, at the base of the Olduvai Event, at about 1.8 m.y. The Miocene/Pliocene boundary is characterized in a similar manner by several biostratigraphical events which, together, approximate the lower boundary of the Pliocene. These include the extinction of *Globoquadrina dehiscens* at about 5.0 m.y.; first evolutionary appearance of *G. tumida* and *C. amplificus*, at about 4.9 m.y.; first evolutionary occurrence of *S. dehiscens*, at about 4.8 m.y. The Miocene/Pliocene boundary is drawn here at 5 m.y.

Nine planktonic foraminiferal datum levels have recently been calibrated to the palaeomagnetic time scale for the past 4 m.y.¹⁴. Seven of these (numbers IX–III) are of Pliocene age. An additional three (XII–X) are suggested here (Table 2) based on more recent data obtained in my laboratory (Table 3) and elsewhere¹⁹. Eleven distinctive calcareous nannofossil datum levels have also been calibrated with the palaeomagnetic time scale⁵⁰. Those of particular importance to this discussion of Pliocene chronostratigraphy are shown in Table 4.

In Fig. 1 the Pliocene calcareous nannoplankton and plank-

tonic foraminiferal zones are placed in a time framework. A summary of the basis for this correlation is presented below.

A continuous, condensed carbonate sequence spanning the interval of latest Miocene to early late Pliocene (>5–<2.8 m.y.) was cored at Site 111 (Orphan Knoll) in the Labrador Sea during Leg 12 of the Deep Sea Drilling Project⁵⁸. Calcareous nannoplankton zones NN12 to NN16 were recorded, each zone having an average thickness of 10–20 cm (ref. 59). Various planktonic foraminiferal datum levels were also observed⁶⁰ and using the extinction level of *Sphaeroidinellopsis* spp. (2.9 m.y.), *Globigerina nepenthes* (3.7 m.y.) and *Globoquadrina dehiscens* (5.0 m.y.) an estimation of age against depth was made for the interval of 2.8 to 5 m.y.

A re-examination of the planktonic foraminifera and calcareous nannoplankton from the critical intervals in Hole 111A has been made. Below 80 cm the sediment shows considerable dissolution and is too strongly condensed for biostratigraphical evaluation. The initial appearances and extinctions of several important taxa and their calibration with the palaeomagnetic time scale are shown in Table 3.

Data from DSDP Leg 13 in the Mediterranean Sea are also pertinent to this discussion. The *Sphaeroidinellopsis* Acme Zone occurs in the basal part of the early Pliocene transgression and was said⁶¹ to correspond to the lowermost part of the *C. tricorniculatus* (NN12) Zone, which spans the interval between 5.7–4.45 m.y.⁵⁰. The Miocene/Pliocene boundary has been shown above to be about 5 m.y. old, so that the *Sphaeroidinellopsis* Acme Zone is actually correlative with the upper part of the *C. tricorniculatus* (NN12) Zone. *C. tricorniculatus* was not recorded below the top of the Messinian evaporites in the Mediterranean Sea⁶². It has been recorded, however, from the basal Messinian marls in the upper part of the type Tortonian section and the lower Pliocene Trubi Formation in Sicily, the basal Piacenzian of the Torrente Arda in northern Italy and in the type Tabianian nearby⁶³ as well as from levels correlated with zones N17–N19 in the Pacific core CAP 38 BP (ref. 64). The absence of *C. tricorniculatus* in the latest Messinian of the Mediterranean is probably due to unfavourable ecological conditions⁵⁰. An overlap in the stratigraphical ranges of *C. tricorniculatus* and *C. rugosus* was observed between 625–545 cm in core CAP 38 BP (ref. 65); this interval is of early Pliocene age, equivalent to the basal part of Zone N19. In the Mediterranean the *C. rugosus* Zone was correlated consistently with the *G. margaritae* *evoluta* lineage Zone or with the base of the concurrent range of *G. margaritae* and *G. puncticulata* at about 4.5–4.6 m.y.⁶¹.

The extinction of *G. margaritae* at the Gilbert/Gauss magnetic boundary (~3.32 m.y.) occurs within the upper part of the *Discoaster asymmetricus* Zone (NN14) in the Mediterranean⁶¹. The North Atlantic data (Leg 12) suggest that the *D. asymmetricus*/*Reticulofenestra pseudumbilica* boundary occurs at about 3.5 m.y. (Table 3), which is slightly older than the extinction level of *G. margaritae* (~3.32 m.y.). The slight discrepancy may be due to the level of resolution we are trying to achieve here (~0.1–0.2 m.y.).

Data from the North Atlantic (Leg 12) and the Mediterranean Sea (Leg 13) agree well with the suggested ages for the various calcareous nannoplankton datum levels (Table 4) with the exception of the *R. pseudumbilica* (extinction) Datum at 2.4 m.y. This level occurs near the bottom of Zone N21⁶⁶ and below Datum V (*Sphaeroidinellopsis* extinction) at the top of the Mammoth Event, about 2.95 m.y.¹⁴, in DSDP core 3/12C/4/2 near Cape Verde⁶⁷, and corresponds to the initiation of continental glaciation in the Northern Hemisphere⁶⁸. Thus, I consider an extinction date of 2.4 m.y. as somewhat too young and, indeed, it was noted⁵⁰ that near the top of its range this species is rare and represented mostly by relatively small specimens. It may be either that the (1) extinction level of *R. pseudumbilica* determined by Gartner⁵⁰ is too high and that the upper part of the range of this species is due to reworking or (2) that the extinction level at 2.4 m.y. is real and that previous determinations on the *R. pseudumbilica*/*D.*

Table 4 Late Miocene/Pliocene Calcareous Nannoplankton Datum Levels

Time (m.y.)	Datum level	First occurrence (FO) Last occurrence (LO)
~1.8	<i>Discoaster brouweri</i>	LO
~2.1	<i>Discoaster surculus</i>	LO
~2.4	<i>Reticulofenestra pseudumbilica</i>	LO
~3.6	<i>Ceratolithus tricorniculatus</i>	LO
~3.95	<i>Discoaster asymmetricus</i>	FO
~4.45	<i>Ceratolithus rugosus</i>	FO
~4.9	<i>Ceratolithus amplificus</i>	FO
~5.7	<i>Discoaster quinqueramus</i>	LO

Data from refs 50 and 51.

surculus boundary are incorrect due to failure to recognize the latest part of the range of *R. pseudoumbilica*. The weight of the evidence cited above suggests that the correct alternative is (1) and so I assign an age of ~3 m.y. to the *R. pseudoumbilica*/*D. surculus* boundary here.

The ages of the *D. surculus*/*D. pentaradiatus* and *D. pentaradiatus*/*D. brouweri* boundaries are estimated at 2.3 and 2.0 m.y. respectively, based on extrapolated sedimentation rates (Fig. 1). Slightly different ages have been estimated by Gartner^{50,51} for these levels (see Table 4) based on a slightly different interpretation of the age of the Olduvai (=Gilsa) Event. It is hoped that additional data will enable us to estimate the ages of these boundaries more accurately. A comparison of the estimated age of several of the biostratigraphical levels discussed is presented in Table 5.

Pliocene Planktonic Foraminiferal Zonation

A three-fold planktonic foraminiferal zonation (N19–N21) has been developed for the tropical Pliocene⁶⁹, but its application to biostratigraphical studies has been criticized by several authors^{70–73}.

Several observations on mid-Pliocene correlations in the Mediterranean region⁶¹ are relevant to our discussion and form the impetus for the Pliocene new zonation suggested below: (1) Zone 21 (*G. tosaensis*) includes the *D. brouweri*, *D. pentaradiatus* and topmost part of the *D. surculus* Zone; (2) the base of Zone N21 lies in the upper part of the *D. surculus* Zone; (3) Zone N20 lies entirely within the *D. surculus* Zone; (4) the top of Zone N19 lies near the base of the *D. surculus* Zone, according to DSDP Leg 7 data. In the Mediterranean

Table 5 Comparison of Pliocene Datum Level Age Determinations

TIME (my)	CITA (Ref. 61)	GARTNER (Ref. 50)	BERGGREN (this paper)
1.6			
1.8			
2.0			
2.2			
2.4		NN 15 / NN 16	
2.6			
2.8			
3.0	NN 15 / NN 16		NN 15 / NN 16
3.2			
3.4			
3.6		NN 14 / NN 15	NN 14 / NN 15
3.8			
4.0		NN 13 / NN 14	NN 13 / NN 14
4.2			
4.4			
4.6		NN 12 / NN 13 = MIOCENE / PLIOCENE BOUNDARY	NN 12 / NN 13
4.8			Sph. dehiscens
5.0		C. amplificus (FO)	G. tumida MIOCENE / PLIOCENE BOUNDARY (C. amplificus (FO) & G. dehiscens (LO))
5.2	MIOCENE / PLIOCENE BOUNDARY		
5.4	(Sph. dehiscens Datum)		
5.6			
5.8		Discoaster quinquerramus (LO)	

the top of the *Sphaeroidinellopsis subdehiscens* interval zone lies near the base of the *D. surculus* Zone and the top of Zone N19 can "be easily correlated with the extinction horizon of *Sphaeroidinellopsis* spp."

The following points can also be made: (1) The difficulty in applying the concept of Zone N20 to biostratigraphical studies in deep sea cores has been expressed by several workers⁷⁴⁻⁷⁸. The definition of Zones N19 and N20 has been emended⁷⁹ so that a distinct interval occurs between the top of N19 and the extinction of *Sphaeroidinellopsis* spp. This latter event occurs near the top of the Mammoth Event, at about 2.95 m.y. and is virtually coincidental with the base of the *D. surculus* Zone⁶¹. But this is also within or near the top of Zone N20. The base of Zone N20, as redefined⁷⁹, is marked by the initial appearance of *G. pseudopima* and not the extinction of *Globoquadrina altispira* as originally suggested⁸⁰. The evolution of *G. pseudopima* occurs before that of *G. tosaensis*.

(2) The base of Zone N21 (*G. tosaensis*) is defined on the basis of the first evolutionary appearance of the nominate form from *G. crassaformis oceanica*⁸¹. This occurs at about 3.0 m.y. and is virtually identical with the extinction level of *Sphaeroidinellopsis* spp.⁸².

(3) The appearance of *Pulleniatina obliquiloculata* in the upper part of Zone N19 (ref. 83) indicates that this level is above the extinction level of *G. nepenthes* (at 3.7 m.y.) which is within N19. Thus, the position of Zone N20 would be somewhere in the interval of 3.7-3.0 m.y. approximately. Actually, experience with deep-sea cores has shown that *Pulleniatina obliquiloculata* appears at or slightly above the *Sphaeroidinellopsis* extinction Datum (about 3.0 m.y.)⁸⁴. Thus, N20 would appear to represent a short interval of time near the *Sphaeroidinellopsis* extinction level and has been estimated to span the interval of 3.3-3.0 m.y.⁸⁴.

The point is that Zone N20, if it does exist, is below Zone N21, older than 3.0 m.y. and below (=older than) the *D. surculus* Zone, the base of which can be dated at about 3.0 m.y. also. Thus, it is probably equivalent to the upper part of the *R. pseudoumbilica* Zone (NN15). Zone 20 is shown in Fig. 1 as possibly equivalent to the upper part of Zone N19 as it is questionable whether there exists a real basis for the consistent recognition of Zone N20.

Several alternative schemes of zonation for the tropical Pliocene sequences could be suggested which would greatly improve the resolution of biostratigraphical correlation while obviating the problems of correlation which now exist because of the dubious nature of N20 and the difficulty in distinguishing Zone N18 because of its extremely short duration (~0.1-0.2 m.y.). The following intervals are suggested as useful in a zonation of the Pliocene: Zone 1—interval from the evolutionary appearance of *G. tumida* from *G. plesiotumida* (4.9 m.y.) to the extinction of *Globigerina nepenthes* (3.7 m.y.); Zone 2—interval from the extinction of *Globigerina nepenthes* (3.7 m.y.) to the extinction of *G. margaritae* (3.4 m.y.); Zone 3—interval from the extinction of *G. margaritae* (3.4 m.y.) to the extinction of *Sphaeroidinellopsis* spp. (3.0 m.y.); Zone 4—interval from the extinction of *Sphaeroidinellopsis* spp. (3.0 m.y.) to the extinction of *Globoquadrina altispira* and *G. multicamerata* (2.8 m.y.); Zone 5—interval from the extinction of *Globoquadrina altispira* and *G. multicamerata* (2.8 m.y.) to the extinction of *G. miocenica* (2.2 m.y.); Zone 6—interval from the extinction of *G. miocenica* (2.2 m.y.) to the extinction of *Globigerinoides obliqua extrema* (1.8 m.y.).

The six zones can be designated as follows: Zone Pl 1: *Globigerina nepenthes*—*Globorotalia tumida* Partial Range Zone. Zone Pl 2: *Globorotalia margaritae*—*Sphaeroidinellopsis subdehiscens* Partial Range Zone. Zone Pl 3: *Globoquadrina altispira*—*Sphaeroidinellopsis subdehiscens* Partial Range Zone. Zone Pl 4: *Globoquadrina altispira*—*Globorotalia exilis* Partial Range Zone. Zone Pl 6: *Globigerinoides obliqua* Partial Range Zone. For convenience the prefix Pl is placed before each zone to indicate that it is of Pliocene age, thus

Pl 1, Pl 2, and so on.

The base of the lowest Pliocene zone, Pl 1 (*Globigerina nepenthes*—*Globorotalia tumida* Partial Range Zone) is equivalent to the base of Zone N18 as the base, in both instances, is drawn on the basis of the first evolutionary appearance of *G. tumida* from *G. plesiotumida*.

The first evolutionary appearance of *G. tumida* is virtually synchronous with the extinction of *Globoquadrina dehiscens* (5.0 m.y.) and these two events may be useful criteria in determining the position of the Miocene/Pliocene boundary. The zonation proposed here provides continuity with the late Miocene part of one of the standard zonation systems now in wide use⁸⁵.

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LETTERS TO NATURE

PHYSICAL SCIENCES

Anomalous Absorption in the Atmosphere for 2.7 mm Radiation

MEASURED values¹⁻⁴ of atmospheric attenuation of electromagnetic radiation in the "windows" at 2 and 3 mm are greater by factors of between two and three than is calculated from monomeric water vapour and oxygen line strengths. This is true for several theoretical formulations of collision broadening. To investigate these anomalies, we have made measurements at 110 and 113 GHz (2.73 and 2.65 mm) of the variation of zenith sky noise temperatures with atmospheric conditions, using Dicke radiometers.

Simultaneous observations at two frequencies provide spectral shape information, whereas a single frequency gives only attenuation magnitude, the variation of which is more difficult to interpret. Although more spectral information could be obtained by scanning over a range of frequencies, millimetre wave techniques are such that the time taken would be too long for atmospheric conditions to remain stable, so the two-frequency method is adopted as an effective compromise. The frequencies were chosen to correspond to

the observations of Straiton and Tolbert^{1,2}, which showed the presence of a spectral feature at 110 GHz. This was shown to be associated with the presence of water vapour, but could not be assigned to it spectroscopically.

From the measurements of sky noise temperatures, values of total atmospheric attenuation were deduced using meteorological data from radiosonde observations taken at Crawley, Sussex. The values of total attenuation were related to the ground level water vapour density obtained from measurements at RSRS since previous experience had shown that the ground level water vapour density was well correlated with the total amount of precipitable water in the path.

A plot of the difference in attenuation at the two frequencies is shown in Fig. 1, as a function of surface water vapour density. A weighted least squares fit to the data yields

$$\alpha(113) - \alpha(110) = 0.53 + 0.002p \quad (1)$$

where the attenuation, α , is in dB, and p is the water vapour density in g m^{-3} . The difference is a measure of the slope of the observed spectrum, and it is compared in Fig. 1 with a calculated value represented by

$$\alpha(113) - \alpha(110) = 0.28 + 0.005p \quad (2)$$

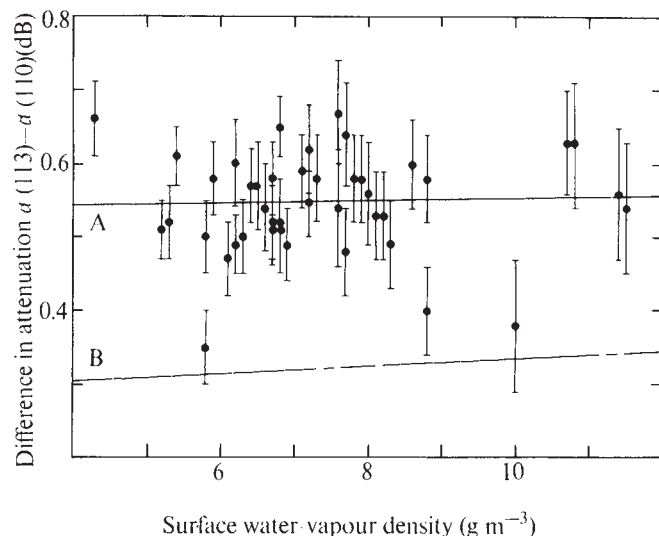


Fig. 1 Difference in attenuation at 110 GHz and 113 GHz as a function of surface water vapour density in g m^{-3} . Curve A is the least squares fit to the observations and curve B (dotted) is the theoretical prediction.

This was obtained using the Van Vleck-Weisskopf line shape for oxygen attenuation, with line-width parameters of $1.59 \text{ MHz torr}^{-1}$ for the 60 GHz lines and $2.14 \text{ MHz torr}^{-1}$ for the 119 GHz line. The former value was obtained by Zhevakin and Naumov⁵, who showed that the Van Vleck-Weisskopf line shape provides better agreement with experiment than the Zhevakin-Naumov line shape. The value used for the 119 GHz line came from other experiments in the present series, details of which will be published elsewhere. For water vapour attenuation, the Zhevakin-Naumov line shape was used, with the half-width parameters calculated by Benedict and Kaplan⁶, for quantum numbers up to 12 and for the frequency range up to 6,000 GHz.

The observed slope is approximately twice that predicted, which supports the conclusion of Straiton and Tolbert that there is anomalous behaviour at these frequencies, but the dependence of the slope on water vapour density does not agree with the earlier results. These indicated a coefficient of -0.06 in equation (1), compared with $+0.002$ in the present results. Some of the data in Fig. 1 apply to measurements made with thin clouds present in the field of the radiometers. The effect of these on the difference in attenuation at the two frequencies is estimated to be not more than 0.03 dB , because liquid water absorption is only 3% higher at 113 GHz than at 110 GHz. But the presence of clouds indicates that some saturated water vapour was in the path, and this could contribute to anomalous absorption.

No reasonable modification of the accepted line-width parameters of water vapour or oxygen allows the present observations to be fitted with existing theories of collision broadening. For example, use of the Zhevakin-Naumov line shape for oxygen⁵ in equation (2), rather than the Van Vleck-Weisskopf line shape, only changes the first term from 0.28 to 0.31 dB . The explanation of anomalous absorption is more likely to be found in the presence of molecular complexes associated with water vapour, for which evidence has been presented from measurements at higher frequencies⁷.

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Recent Freezing Level Changes and Climatic Deterioration in the Canadian Arctic Archipelago

It is well established that climatic fluctuations which are global or hemispheric in extent are most pronounced in high northern latitudes, particularly around the North Atlantic¹⁻⁴. Recent evidence also suggests that certain sectors of the Arctic are extremely sensitive to small climatic shifts and as a result may undergo visible changes in the landscape⁵⁻⁷. Here I report on an analysis of upper air data for the Canadian Arctic archipelago which indicates that marked changes in freezing level heights have occurred during the past two decades as a result of changes in atmospheric circulation across the area.

Over most of the Canadian Arctic, surface temperatures rarely rise above 0°C from September to May. The freezing level (the 0°C isothermal surface) reaches a maximum elevation in July. A decrease in the elevation of the freezing level during summer months can therefore greatly decrease the area of snow and ice affected by melting.

Bradley and Miller⁵ suggested that a significant climatic deterioration began on Baffin Island about 1960. The freezing level data indicate that the critical change probably occurred around 1962-1964. Table 1 shows average July freezing level elevations for two nine-year periods, 1955-1963 and 1964-1972, based on an analysis of daily records (no data available prior to 1955). July freezing levels have been much lower at all stations in the area during the past nine years. A similar pattern

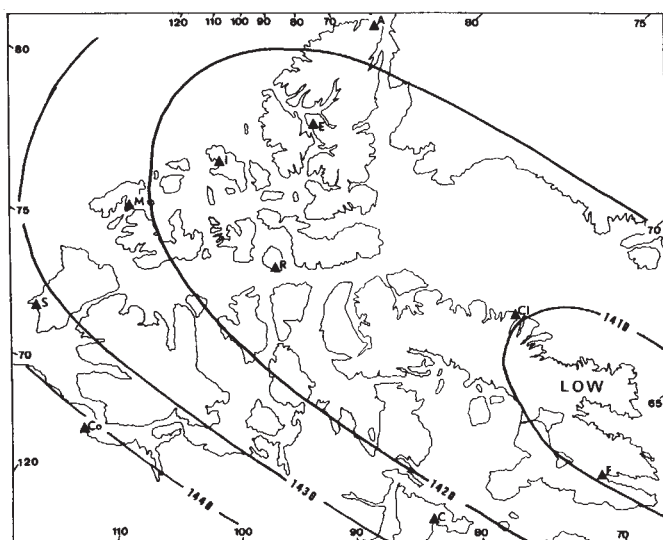


Fig. 1 Average height of the July 850 mbar surface, 1955-63, in geopotential metres ($1 \text{ gpm} \approx 0.98 \text{ m}$). Letters refer to upper air stations mentioned in text. A=Alert; C=Clyde; C=Coral Harbour; Co=Coppermine; E=Eureka; F=Frobisher Bay; I=Isachsen; M=Mould Bay; R=Resolute; S=Sachs Harbour.

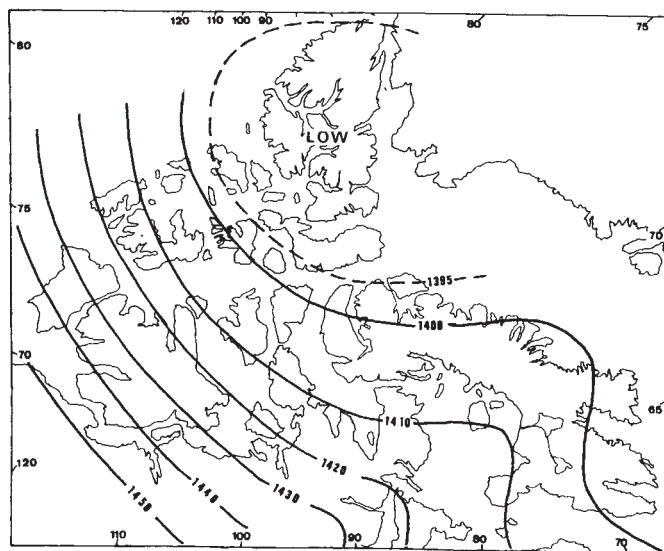


Fig. 2 Average height of the July 850 mbar surface, 1964-72, in geopotential metres.

1961 as a result of anomalously warm ocean temperatures in the central northern Pacific Ocean which had the effect of amplifying the standing long wave pressure pattern in the Northern Hemisphere. The observed displacement of the main centre of the eastern Canadian trough north and west is consistent with Namias's suggestions. The abrupt changes in freezing levels around 1962-64 reflect a lag in response to the initiation of this new circulation regime.

The large changes in freezing level height since 1963 have produced significant glaciological responses in the Canadian Arctic. Observations made in 1967 in the United States Range, central Ellesmere Island, led Hattersley-Smith⁹ to conclude that the summers of 1963-66 "were the coldest sequence of summers since before 1925 . . . the equilibrium line (ELA) on the glaciers had been lowered to approximately 900 metres from a mean of about 1,200 metres in the years 1957-63". This fall in ELA by 300 m is similar to the drop in freezing level at Alert and Eureka (Table 1). Similarly, the ELA on the White Glacier, central Axel Heiberg Island, has been more than 400 m lower since 1963-64 than in the four years 1959-60 to 1962-63 (ref. 10). Again a similar fall in freezing level height over this area has been noted. Finally, a recent study of sea-ice extent in Baffin Bay and Davis Strait shows that a very marked

Table 1 Average July Freezing Level Heights, 0000 GMT.* Heights in Geopotential Metres

Station	Latitude	Longitude	(a) Freezing level 1955-63	(b) Freezing level 1964-72	Change (b-a)	Student's <i>t</i>	Statistical significance
Alert	82° 30'	62° 20'	1,230	900	-330	4.9	0.1%
Clyde	70° 27'	68° 33'	1,620	1,280†	-340	4.5	0.1%
Coppermine	67° 49'	115° 05'	2,280	2,090‡	-190	2.9	0.5%
Coral Harbour	64° 12'	83° 22'	2,100	1,860	-240	3.7	0.1%
Eureka	80° 00'	85° 56'	1,500	1,150	-350	7.2	0.1%
Frobisher Bay	63° 45'	68° 33'	2,010	1,710	-300	4.9	0.1%
Inuvik	68° 18'	133° 29'	2,500§	2,370	-130	2.2	2.5%
Isachsen	78° 47'	103° 32'	1,220	710	-510	7.3	0.1%
Mould Bay	76° 14'	119° 20'	1,310	1,150	-160	2.1	2.5%
Resolute	74° 43'	94° 59'	1,490	1,150	-340	4.7	0.1%
Sachs Harbour	71° 59'	125° 17'	1,790	1,640	-150	1.8	5.0%

* July 1955 soundings taken at 0300 GMT.

† 1964-70 inclusive.

‡ Data 1970-72 inclusive from Cambridge Bay (69° 06' N, 105° 07' W).

§ Data 1955-60 inclusive from Aklavik (68° 14' N, 135° 00' W).

|| 1956-63 inclusive.

is apparent for the summer months as a whole. A maximum freezing level depression of 510 geopotential metres occurred at Isachsen in the northwestern archipelago with other large decreases in a broad belt north to Eureka and Alert and south-east to Resolute, Clyde and Frobisher Bay.

Mean pressure contour maps for the same periods (Figs. 1 and 2) indicate that during the former period the area was dominated by a trough of low pressure over Baffin Bay and southern Baffin Island. In the subsequent period the principal low pressure centre occurs to the northwest with the south-eastern Arctic dominated by a ridge of high pressure. Pressure gradients over the archipelago are also much stronger in the latter period indicating increased movement of air across the region, particularly from the north. A similar pattern of circulation change is observed at the 700 mbar level. The low pressure centre of 1964-72 is located precisely where the most significant falls in freezing level have occurred (Table 1). Increased advection of cool air across Baffin Island during the latter period accounts for the relatively large falls at Clyde and Frobisher Bay. Relatively small changes over the south-western archipelago reflect little change in the circulation over that area.

The large changes in summer freezing levels across the area thus seem to be the result of changes in synoptic conditions. Namias⁶ suggests that a "new climatic regime" began late in

change towards more severe ice conditions occurred in 1963 (ref. 11). Between 1952 and 1960 there were only four years when significant areas of ice were observed in September whereas "in the 1960s there was only one year in which ice was not present in September, and five years in which it survived until October". Such a deterioration clearly reflects the lower temperatures and increase in northerly flow over the area during the past decade.

In conclusion, the Canadian Arctic has recently experienced a significant climatic deterioration. Any further fall in freezing level heights or the persistence of recent conditions will undoubtedly result in increased glacierization throughout much of the eastern and northern upland area. This indicates the importance of carefully monitoring climatic conditions in the Canadian Arctic archipelago over the next few years.

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Compensation Law in Thermodynamics and Thermal Death

THE isokinetic relationship or, more generally, the compensation law, a linear relation between enthalpy and entropy, has been debated for many years. Evidence for it occurs in phenomena such as chemical reactions, solubility, evaporation of metals, catalytic processes, thermal denaturation of macromolecules, thermal killing of unicellular organisms, and semiconductor processes. Such a widespread occurrence advises avoidance of *ad hoc* explanations, and suggests a common denominator on a thermodynamic or statistical mechanical basis.

Objections raised to the validity of a compensation law fall into four categories, namely, (1) that there are inadequate data for a sufficient range of temperatures and number of Arrhenius lines; (2) that logarithmic compression accidentally produces an apparent compensation law; (3) that biased sets of data are chosen; and (4) that such a law cannot, at present, be derived from first principles of statistical mechanics, or thermodynamics. The objections recently raised by Banks *et al.*¹ fall into categories (2) and (3). They are correct to point out the dangers in simply plotting the measured values of $\Delta H^\ddagger$ from Arrhenius plots against the calculated values of $\Delta S^\ddagger$ from the transition rate theory equation, because both are proportional to the logarithms of the rate constants. Also, because of experimental bias in favour of a comfortable range of reaction rates, and the biological bias that death rates of unicellular organisms should be measured in times less than cell division times, one may accidentally pick out a subset of protein denaturation rates and death rates which provide a subset of $\Delta H^\ddagger$ and $\Delta S^\ddagger$ values falling in the same narrow strip of the $\Delta H^\ddagger$ - $\Delta S^\ddagger$ plane.

An entirely different argument inverts the argument of Banks *et al.* This refers to Arrhenius plots the slopes of which yield very high activation enthalpies. For reaction rates, activation enthalpies greater than 50 kcalorie mol⁻¹ are fairly common. Conventional pre-exponential factors cannot possibly be large enough to offset these extremely small exponential values in order to yield the measured rates. Thus, in order for such reaction rate constants to be measurable, there must be another, positive, exponential term added to the Arrhenius equation. If the activation enthalpy can be varied over a wide range of values, this positive exponential factor must also vary with it and thus this factor must also be a function of the activation enthalpy and must be independent of temperature. Such a term is $\exp(\Delta S^\ddagger/R)$ where $\Delta S^\ddagger$ is proportional in some way to $\Delta H^\ddagger$. This is a compensation effect. The simplest, though not necessarily correct, proportionality is a linear relation $\Delta S^\ddagger = a\Delta H^\ddagger + b$. This is one form of a compensation law. We conclude from this that the only physically detectable processes of high activation enthalpies are those where a positive exponential factor operates, and if the activation enthalpies vary over a sufficiently wide range, the compensation law must be valid. If the enthalpies

only vary over a small range one must exercise extreme caution and apply a rigid criterion of validity before accepting or rejecting the hypothesis of the operation of a compensation law². For a very wide range of activation enthalpies, empirically determined from good Arrhenius plots, one must accept the operation of a compensation law. Because the thermal denaturation of proteins and the thermal inactivation or death of unicellular organisms contain a majority of cases of very high activation enthalpies, but also include lower ones, these must be assumed to be compensation law processes³.

There are many ways to evaluate a and b in the linear $\Delta S^\ddagger$, $\Delta H^\ddagger$ relation. The simplest is to plot $\Delta S^\ddagger$ against $\Delta H^\ddagger$ for the appropriate series. Logarithmic compression now minimizes the fluctuations in the rate constant data. Thus $T_c (=1/a)$ and b can be evaluated fairly accurately and used for correlational purposes³. The range of variation of $\Delta H^\ddagger$ must be larger than about 50 kcalorie mol⁻¹ if this method is used to establish the validity of the compensation law. The data for protein denaturation and thermal deaths cover a range of $\Delta H^\ddagger$ of about 150 kcalorie mol⁻¹.

We agree that large values of $\Delta H^\ddagger$ are likely to be associated with complex processes, because unit steps do not involve so much enthalpy. Protein denaturation is certainly a complex process. Such processes, however, can also be described by the absolute theory of rate processes. The problem of combining unit steps into a complex process, which in turn also looks like a unit process, has also been treated by one of us (G. K., unpublished). The theory of rate processes can be used, contrary to Banks *et al.*, in the gas, liquid, or solid phases. In complex cases, we may not be able to assign an origin to $\Delta H^\ddagger$ and $\Delta S^\ddagger$, but this does not mean that these quantities are meaningless. Although there is a difference in practice between "unit steps" and complex processes, we see no difference in principle, because "unit steps" (that is, breaking of one chemical bond) are themselves due to multiple steps of additions of vibrational energy.

Even if the compensation law for complex processes were not quantitatively accurate, there must be a general trend that larger values of $\Delta H^\ddagger$ are associated with larger values of $\Delta S^\ddagger$. As $\Delta H^\ddagger$ increases for a reaction series, this increasing enthalpy must be shared out among an increasing number of units. The larger the number of units, the larger will be the density of states associated with the activation. This leads to the rough proportionality between $\Delta H^\ddagger$ and $\Delta S^\ddagger$. This line of reasoning leads to a statistical mechanical interpretation of the compensation law and of the compensation temperature (G. K. and Goklany, unpublished).

Banks *et al.* state that because bacterial cells are either alive or dead, death is necessarily a first order kinetic process, as in radioactive decay. That is not true. Plots of the logarithm of the mortality rate as a function of time sometimes exhibit an initial shoulder before a straight line portion. This may be attributed to multiple hit requirements, as in radiation effects, or repair mechanism of some damage. We have excluded these cases. Multicellular organisms including *Drosophila*, mammals and man may also be either alive or dead. In these cases, it is known that the kinetic rate constants are time dependent, following a Gompertzian function. It is not yet known at which stage in the transition from unicellular to higher multicellular organisms, the kinetics change from the exponential to the Gompertzian.

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Thermodynamic Compensation Rule

Banks, Damjanovic and Vernon¹ have proposed that the thermodynamic compensation rule which is claimed to apply to the denaturation of proteins^{2,3} is the result of an experimental artefact and thus of no physical significance. In particular, by taking logarithms of the unimolecular rate equation, they obtain

$$\log C - \log kT/h = \Delta S^\ddagger/2.303R - \Delta H^\ddagger/2.303T \quad (1)$$

where C is the rate constant of the reaction. Provided that $\log C$ does not vary very much, for a small range of temperatures the left side of equation (1) is approximately constant so that $\Delta S^\ddagger$ and $\Delta H^\ddagger$ are linearly related. But the real point of physical significance is not so much that $\Delta S^\ddagger$ and $\Delta H^\ddagger$ are linearly related, but that $\log C$ should vary so very little with $\Delta H^\ddagger$. In the particular case they consider, this is the result of the experimental difficulty of measuring more than a small range of values of C and T so that only those materials which apparently obey the compensation law are susceptible to investigation.

The compensation law is also observed, however, in the electrical conductivity of organic semiconductors⁴ and amorphous semiconductors⁵. In those cases the measurable range of conductivity, σ , extends over at least twenty orders of magnitude so that any constancy of σ at a particular temperature would be of real physical significance. In the cases cited this critical temperature is much higher than the experimental range of temperatures investigated, so that at the experimental temperature, the left side of equation (1) is no longer constant. In the case of amorphous semiconductors the critical temperature is greater than 1,000° C. No doubt with a suitably wide and uncorrelated variation in $\Delta H^\ddagger$ and σ it would be possible to group together materials such that they apparently obey a compensation law. It is, however, physically significant that the groupings so obtained correspond to the groupings that would have been made on chemical or structural grounds.

Although a number of explanations of the compensation law for conductivity have been proposed⁶⁻⁹, there remains the possibility that this is also an experimental artefact¹⁰. But it is clear that the particular explanation proposed by Banks *et al.* cannot be appropriate in this case.

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Compensation Effect and Experimental Error

Banks, Damjanovic and Vernon¹ suggest that the observed compensation effect in the rates of some biological processes might be wholly artefactual. Their argument is one which would also apply to systems of purely chemical interest and raises the question of whether, in the inevitable presence of experimental errors, a true compensation effect is ever observable.

I use the term compensation effect to describe the systematic variation of the pre-exponential factor, A , with the activation energy, E , in the Arrhenius equation

$$k = A \exp(-E/RT) \quad (1)$$

often observed for a series of similar reactions. Here k is the specific rate constant, T the temperature and R the gas constant. When a variation is observed, it is usually² found to have the form

$$\log A = \frac{E}{2.303RT_s} + \log k_0 \quad (2)$$

the parameter T_s has units of temperature and corresponds to the temperature at which all the reactions of the series proceed at the same rate, k_0 .

Cremer³ discussed the possibility that compensation effects may arise out of experimental errors. For simplicity I consider separately the effect of uncertainties in E and k on $\log A$. Rearranging equation (1) and taking logarithms

$$\log A = \log k + \frac{E}{2.303RT} \quad (3)$$

For an uncertainty δE in E there will be an associated uncertainty $\delta \log A$ in $\log A$:

$$\log A \pm \delta \log A = \log k + \frac{E \pm \delta E}{2.303RT} \quad (4)$$

Comparing this with equation (2) it is seen that the range of

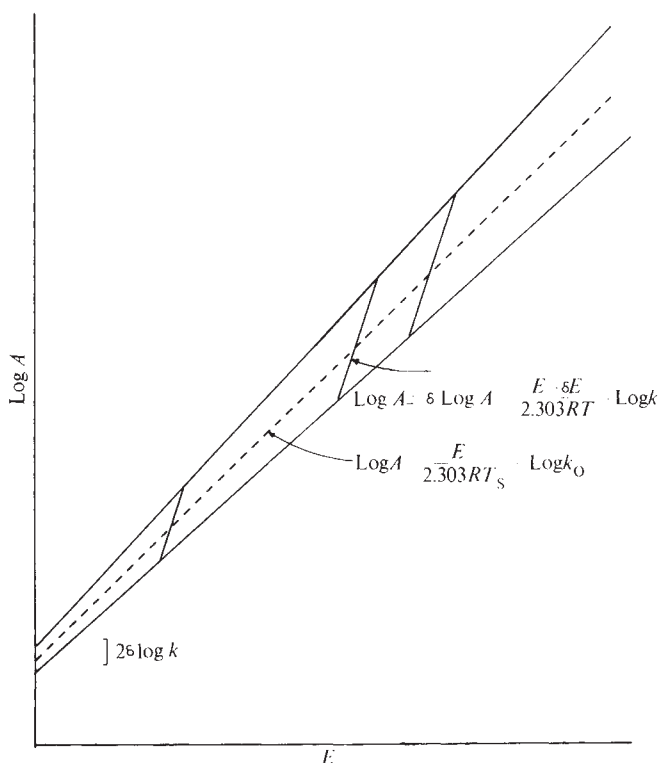


Fig. 1 Range of expected compensation effect in the presence of experimental error.

values of $E \pm \delta E$ and $\log A \pm \delta \log A$ are related by a compensation effect with $T_s = T$. But the uncertainty in $\log k$ of $\delta \log k$ has an associated uncertainty in $\log A$ of identical magnitude independent of E . In a plot of experimentally observed values of $\log A$ and E for a particular reaction, therefore, the experimental temperature determines the slope and the uncertainty in $\log k$ determines the scatter. The fit to the compensation effect plot is independent of the uncertainty in E , as was found by Banks, Damjanovic and Vernon¹, but the spread of activation energies covered by it is only $2\delta E$. Because it is k which is measured experimentally and $\delta \log k$ which is responsible for the scatter, compensation effect plots of this kind would usually appear to be obeyed very well.

I now consider the experimentally observed compensation effect for a series of reactions in which E and A are intrinsically related by equation (2). Taking δE and $\delta \log k$ as the uncertainties in E and $\log k$ as before,

$$\log A \pm \delta \log A = \frac{E}{RT_s} \pm \frac{\delta E}{RT} + \log k_0 \pm \delta \log k \quad (5)$$

This represents an area in the compensation effect plot bounded by the envelope of all of the compensation effects due to errors in E and $\log k$ for all the individual reactions. The shape depends on the relative magnitudes of T and T_s in equation (5) and on the relation between δE and E . In Fig. 1 I show the case where δE is proportional to E . Clearly as T approaches T_s the fit to equation (2) improves, and when $T \neq T_s$ the magnitude of δE becomes important.

I conclude that if the range of an experimentally obtained compensation effect exceeds twice the uncertainty in the activation energy then there probably exists an intrinsic relation between A and E . In the absence of large systematic errors the parameter T_s is obtained from the slope of the compensation effect plot. Only the quality of the plot is dependent on the magnitudes of the errors and it is the uncertainty in E which assumes greatest importance at temperatures much different from T_s .

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REPLY to Boon and to Harris: We are well aware that the so-called compensation law behaviour has been observed in the case of the electrical behaviour of semiconductors, and as we indicated, may not be wholly artefactual in some cases in physical organic chemistry. We were merely concerned to show that the experimental data on the denaturation of proteins and the thermal death of viruses and microorganisms cannot be used to adduce compensation law behaviour in these cases. The linearity of the plot of $\Delta H^\ddagger$ against $\Delta S^\ddagger$ arises wholly from the near constancy of the left hand side of the equation used in calculating $\Delta S^\ddagger$ from values of $\Delta H^\ddagger$ which vary between 10 and 200 kcalorie mol⁻¹. It is unlikely that the uncertainty in the experimentally determined activation energies for protein denaturation is greater than half the range over which compensation law behaviour is observed so the analysis put forward by Harris does not seem to cover all cases where compensation law behaviour is artefactual.

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Compensation Effect in Heterogeneous Catalytic Reactions including Hydrocarbon Formation on Clays

A SERIES of comparable reactions is said to exhibit compensation behaviour when the Arrhenius plots show systematic variations of the activation energy (E) and the pre-exponential factor (A) according to the expression

$$\log A = bE + c \quad (1)$$

Many examples of such behaviour have been reported for diverse reaction systems, but hitherto no explanation of the effect has been generally accepted. Indeed, it has been suggested¹⁻³ that obedience of data from a sequence of reactions to equation (1) may arise through the method of analysis of the kinetic measurements or it may be an artefact. But before compensation behaviour is dismissed as a phenomenon of little theoretical significance, we suggest that in at least one field, heterogeneous catalysis, obedience to equation (1) may be considered to result from a useful mechanistic model. Many examples of compensation behaviour among reactions catalysed by solids have been reported and Bond⁴ has discussed the theoretical implications. The present preliminary report describes a further sequence of surface rate processes which exhibit the compensation effect and proposes a model to explain these observations.

Alcohols and hydrocarbons react⁵ on montmorillonite surfaces to yield a characteristic mixture of hydrocarbon products in which *iso* and *anteiso* alkanes predominate. The mechanism of these reactions, proceeding under conditions of possible primary product and water vapour readsorption⁵, includes polymerization, cracking, isomerization and hydrogen transfer steps. We have now completed a study of the reactions of *n*-dodecanol and of stearic acid on the clay minerals illite, kaolinite and montmorillonite. These reactions yielded product hydrocarbon mixtures similar to those referred to above⁵ and will be described elsewhere. The present report is restricted to the consideration of two particular observations for the six reactant mixtures studied: (1) The A and E values exhibit compensation behaviour (Fig. 1); (2) kinetic data, measured for comparable reaction rates, were obtained at similar temperatures (within the approximate range 420–550 K).

From comparison of the product mixtures, we conclude that the reactions to which Fig. 1 refers proceeded by comparable mechanisms involving⁵ the rearrangement of similar mixtures of hydrocarbon fragments bonded to the clay surfaces. In spite of crystallographic differences between the three mineral phases, the catalytic properties of the active surfaces, composed of somewhat different arrays of oxide and hydroxide ions⁶, were closely similar. Significantly, these similarities include the appearance of detectable catalytic activity at temperatures slightly below the range of onset of bulk dehydroxylation. Accordingly, we associate the participation of clays in reactions of organic compounds with the reactivity and mobility of active constituents of the mineral surface. Such surface species undoubtedly include protons and hydroxyl groups which are involved in the dehydration reaction; water loss, in common with many reactions of solids, probably proceeds preferentially at crystal surfaces. The same groups

may also be expected to participate in the dissociative adsorption and subsequent surface catalytic rearrangements during which *n* dodecanol, or stearic acid, was converted to hydrocarbons. We selected 2-methyl hexane as the representative product for which kinetic data are reported here (Fig. 1).

To discuss a possible mechanistic explanation of compensation behaviour, we write the expression for the rate of product (*P*) formation from a heterogeneous reaction in the form⁴

$$dP/dt = \nu \sigma S_H S_C \exp(-E'/RT) \quad (2)$$

where ν is a surface vibration frequency, σ includes entropy and orientation factors and S_H and S_C are the effective surface concentrations of the hydrogen and the hydrocarbon fragment which react on the catalyst in the step which controls the rate of product formation. We wish to consider the significance of the magnitude of $S_H S_C$ on the observed kinetic behaviour. A temperature dependence of this term, across the range for which rate constants are measured, must influence the value of the apparent activation energy. For example, the value of E determined for a reaction in which the change in the magnitude of $S_H S_C$ is the same as that resulting from the exponential temperature dependence is 0 or $2E'$, depending on the direction of the change. It is reasonable to suppose that such changes in the availability of surface reactant species with temperature do occur, due to surface equilibria, including the heat of reactant adsorption. It may be expected therefore that there would be different rates of variation of $S_H S_C$ with temperature in a sequence of different reactions proceeding by similar mechanisms within a comparable temperature interval and having equal values of ν , σ and E' . Quantitative consideration of the effect of such variations in the availability of those species which react in the rate limiting step indicates that a compensation effect will occur for the measured values of A and E . Further, the range of values of A derived from such considerations extends beyond the limits expected for a simple surface reaction, but which have been found in experimental measurements (for example, $10^{22} < A < 10^{34} \text{ mol m}^{-2} \text{ s}^{-1}$, Fig. 1). In the following discussion we consider application of this model to the compensation effect in heterogeneous catalysis.

The estimation of the concentrations of particular surface species included in equation (2) (S_H and S_C) may be difficult, if not completely inaccessible to experimental measurement, because the identification of the participants in the rate limiting step of a surface process is not always possible. Active intermediates may occupy only a small fraction of the catalyst surface⁷. Heterogeneous reactions often proceed at a temperature range within which there may be extensive dissociative adsorption of the reactant molecules. Surface coverage may be incomplete due to desorption of the reactants and/or products. For example, during hydrocarbon cracking reactions on nickel (a series of reactions which exhibits⁸ compensation behaviour) there may be dissociation of chemisorbed methane (and other hydrocarbons) and hydrogen desorption (ref. 8 and articles cited therein). Thus the complexity of the surface dissociation processes and interdependent equilibria in many systems of interest makes it difficult, if not impossible, to estimate meaningfully S_H and S_C . The majority of chemisorption measurements have, for obvious reasons, been concerned with processes occurring below the temperature range of rapid catalytic reactions. There is no reason for an *a priori* assumption that the product $S_H S_C$ should invariably remain constant within the conditions used for an activation energy measurement. It seems more probable intuitively that there would be changes in surface equilibria, perhaps involving exponential variations of S_H and of S_C with reciprocal temperature.

We emphasize that S_H and S_C represent effective concentrations of surface species; this must include the influence of surface mobility on the reaction frequency factor. At low temperature each reactant species is firmly anchored at a

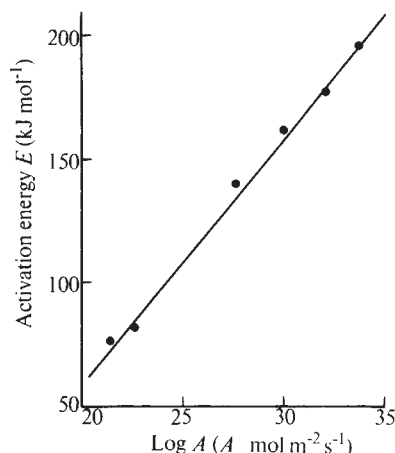


Fig. 1 Compensation plot for hydrocarbon formation reactions on clays. Values of A and E obtained from measurements of the rates of 2-methyl hexane evolution during the reactions of *n*-dodecanol and of stearic acid on illite, on kaolinite or on montmorillonite.

particular site, so that the probability of a collision leading to reaction is negligibly small. On raising the temperature, the probability of contact between reactants is increased by surface migration, but the meaningful estimation of a collision number is impracticable. Surface movements, representing a redistribution of chemisorption bonds, must be an important factor in the reactivity of adsorbed species. We have shown that the catalytic properties of the clays become significant in the temperature range immediately below that at which interaction between surface groups leads to mineral dehydration. Thus there is here an association between surface reactivity and compensation behaviour.

Compensation effects are usually described for a sequence of chemical reactions in which one or more factors, such as reactants, products, catalysts and possibly the rate limiting process, are common to each reaction within the group which exhibits the effect. Examples include hydrocarbon formation reactions on clays (Fig. 1), hydrocarbon cracking reactions on nickel⁸, methane exchange reactions on alloys⁹, the decomposition of formic acid on silver¹⁰ and others⁴. The chemical changes occurring in the reactions of each group are closely comparable, but surface equilibria, and thus concentrations of the intermediates, may be influenced (through the heat of adsorption) by catalyst structure (clays, alloys⁹, and surface structure of silver¹⁰) or the different organic reactants (cracking on nickel⁸). Pétermann¹¹ has ascribed compensation effects in the desorption of hydrogen from metals to temperature variations of transmission coefficients.

Compensation behaviour can therefore be provided with a mechanistic basis in which there are different variations in the temperature dependence of the effective concentrations of the surface species which interact during the rate-limiting step for product formation in a sequence of comparable reactions. Such changes in reactant availability result from differences in surface equilibria and heats of adsorption, reactant dissociation, distribution and mobility. Features of this model, involving variations of the concentrations of intermediates, may also be applicable to the theoretical consideration of homogeneous reactions which exhibit the compensation effect.

The data in Fig. 1 extend previous kinetic studies of reactions which may participate in petroleum genesis^{12,13}. Two general points may be made:

First, the compositions of the mixtures of product hydrocarbons from all six reactant mixtures were closely comparable, thus indicating the conversion of organic compounds to hydrocarbons by the same mechanism (discussed previously⁵) on the different mineral surfaces. The catalytic activity is

therefore associated with similar surface sites on the several aluminosilicates.

Second, the Arrhenius parameters for hydrocarbon formation from the different reaction mixtures showed considerable variations, and the system quantitatively considered in the geochemical context¹³ (*n*-dodecanol and montmorillonite) showed the largest dependence of rate on temperature. Thus, if the extrapolation used¹³ to estimate hydrocarbon formation in sedimentary deposits at lower temperatures is applicable, this particular mixture of reactants must be regarded as the least active of the series currently investigated. The conclusion that clay/organic compound mixtures provide a feasible route for the catalytic production of hydrocarbons in nature is further supported by the present observations. Indeed, the indications from the kinetic study are that illite and kaolinite may be more active catalysts than the montmorillonite first studied.

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BIOLOGICAL SCIENCES

Release of ATP from Rat Motor Nerve Terminals

INVESTIGATION of the adrenal medulla^{1,2} and the *Torpedo* electroplax³ suggested that ATP plays some part in the storage and release of chemical transmitters. The fluid bathing contracting amphibian muscle contains ATP, but this ATP was thought to be released from the muscle⁴. We present here evidence that ATP is released from motor nerve terminals on indirect stimulation of a mammalian nerve-muscle preparation.

To detect ATP we used our bioluminescence injection-housing system, described elsewhere⁵. The sensitive photomultiplier utilizes a bialkali photocathode and is capable of detecting as little as 9.2×10^{-11} g ATP per ml saline when exposed to crude commercial firefly lantern extract (Sigma). As Fig. 1 shows, opening the shutter of the housing system (Fig. 1, S) reveals the resting luminescence of the extract (Fig. 1, L) due to endogenous ATP. On injection of the indicated concentrations of ATP in 250 μ l of solution (Fig. 1, arrow) a chloride-induced inhibition of the resting luminescence of the extract occurs followed by the classical light emission reaction⁶. The peak of light emission serves as a linear quantitative assay for ATP. The baseline for measurements of light emission was taken as the inhibited level after injection of ATP-saline (Fig. 1, arrow). Our particular assay enables

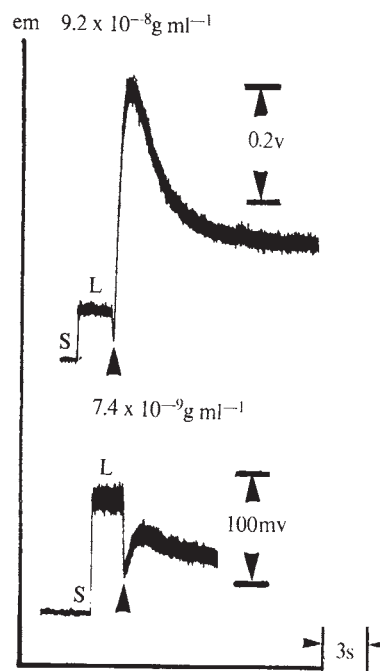


Fig. 1 Chart record of the light emission after injection of 250 μ l of physiological saline solutions of ATP into 430 μ l of firefly lantern extract. Additional details in text.

ATP to be distinguished from ADP and from other nucleoside triphosphates, as each nucleotide has a different temporal pattern of light emission.

Our preparation was the left phrenic nerve-hemidiaphragm of white albino rats, carefully dissected to avoid cut muscle, pinned in a lucite chamber and stimulated indirectly through a polyethylene suction electrode. Preparations were bathed

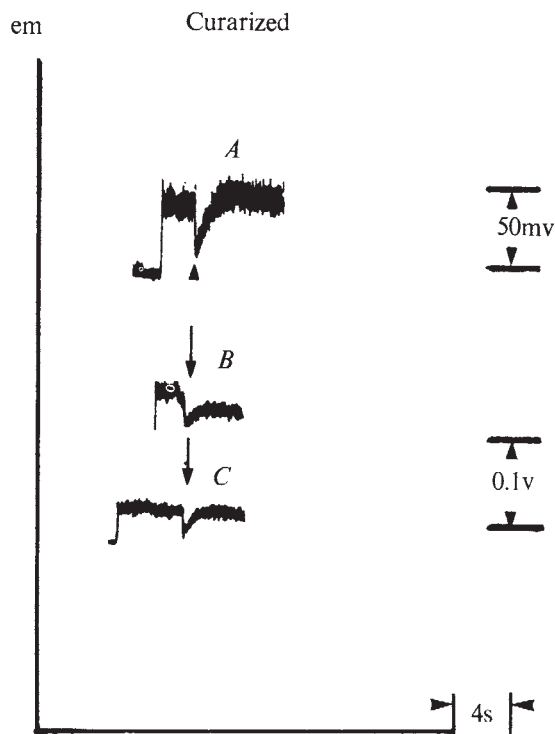


Fig. 2 Chart records of light emission after injection (arrows) of 250 μ l samples of saline taken from the solution bathing a curarized preparation which was either indirectly stimulated (A, C) or rested for 20 min (B). In (A), stimulation was at 8.5 Hz for 15 min and the preparation was soaked for 5 min before the sample was taken. In (C), stimulation was for 20 min at 9 Hz. Note that sensitivity for B and C is half that for A. The light emission recorded in A is equivalent to that produced by approximately 6×10^{-7} g ml^{-1} ADP.

in the modified physiological saline of Liley⁷ at room temperature.

Preliminary investigations on contracting muscle using a protocol similar to that of Boyd and Forrester⁴ (washing for 10 min, nerve stimulation at 2 Hz for 25–30 min and soaking for 5 min) yielded essentially the same quantity of ATP in the perfusion fluid as was detected in their amphibian studies. It was found, however, that in the rat preparation the nucleotide efflux for the 35–40 min period investigated was actually a washout of extracellular ATP and therefore not related to the contractile process. Indeed, in none of seven stimulus-control periods in four separate preparations was there any stimulus-specific release of ATP detectable in the bath of the contracting diaphragm, even if the preparation was first perfused for 1–2 h before nerve stimulation at 2 Hz.

Release of ADP, associated with nerve stimulation in the absence of contraction, however, could be detected. Figure 2 shows the results from a preparation curarized to a level at which no end-plate potentials could be detected. In this instance, approximately six times as much ADP was present in the bath after stimulation (Fig. 2A and C) as in the control period (Fig. 2B). The perfusion fluid at the time of assay was devoid of ATP.

It seemed probable that the absence of ATP in the effluent indicated the presence of nucleotidases activated by Ca^{2+} and Mg^{2+} (refs. 8 and 9). This hypothesis was supported by the results of two experiments in which, when the normal bathing 2 mM (Ca^{2+}) was raised to 5 mM, neither ATP nor ADP was detectable in the bathing fluid of the preparation. To minimize this complication, preparations were bathed in solutions with no added Mg^{2+} and only 0.1 mM Ca^{2+} . ACh release was then effected by exposure to a solution made hyperosmotic by the addition of sucrose and by nerve stimulation. In such conditions of hypertonicity these preparations show a large increase in spontaneous ACh release¹⁰. As Fig. 3 hy shows, 30 min after the beginning of perfusion with the hyperosmotic solution (500 mosM), ATP efflux was increased to a constant level 3.1 times control values. A similar increase in spontaneous ACh release would be expected¹⁰ at this time. On indirect stimulation (Fig. 3 hy, Stim 14 s⁻¹), a 29% increase in the amount of ATP in the bath was observed.

These results are strongly indicative of ATP release from nerve terminals. Two additional lines of evidence support this suggestion. First, when preparations were bathed in less hyperosmotic solutions (400 mosM), only a doubling of ATP

efflux was observed. On stimulation, however, a larger increase in ATP release occurred in the 400 mosM solutions (mean of 43% in three experiments) than in the solutions of higher osmolarity (Fig. 3). This difference would be expected if the nucleotide were associated with transmitter release, for in the presence of 500 mosM solutions there is a decline in the amount of ACh released by nerve impulses after exposure for 8–10 min¹⁰. Second, when the experiments were repeated in the absence of Ca^{2+} , which would prevent ACh release by nerve impulses, no increase in ATP efflux occurred on nerve stimulation in the hyperosmotic solutions.

Additional experiments suggested that the ATP was not derived from nerve above the terminal; as shown in Fig. 3 repetition of the experiments on phrenic nerves revealed no difference in ATP release in any of the experimental situations. This does not, however, eliminate the possibility that diaphragm ATPases were inhibited in hyperosmotic solutions or that some non-specific effect of hypertonicity was contributing to the increase in ATP efflux illustrated in Fig. 3 hy.

All preparations in the hyperosmotic studies were perfused for 2.5 h before the assay of effluents, and contracted in response to nerve stimulation when returned to a normal bathing solution at the end of an experiment.

We suggest that ATP released by nerve stimulation comes from nerve terminals. This ATP could play some part along with protein in the complexing of ACh in the storage vesicle and may also have a trophic function³.

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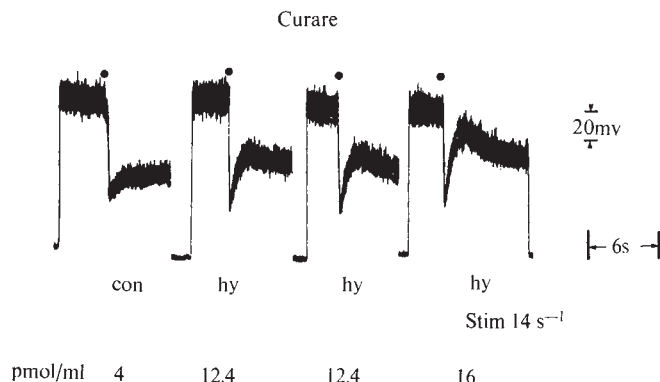


Fig. 3 Chart records of light emission evoked by samples of the fluid bathing a preparation exposed to either a hyperosmotic solution (hy) or to a hyperosmotic solution and nerve stimulation (hy, Stim) in the absence of added Mg^{2+} and 0.1 mM Ca^{2+} . The preparation was curarized. Actual concentrations of ATP in the bath are indicated beneath each record. Control (con) represents ATP efflux before the addition of hyperosmotic solutions; hy, two consecutive 10 min determinations of bathing fluid ATP concentration after the preparation was first exposed to hyperosmotic (500 mosM) solutions for 30 min; hy, Stim 14 s⁻¹, bathing fluid following nerve stimulation at 14 Hz for 10 min. Dots indicate the time of sample injection. Additional details in text.

Thermal Synthesis of Amino Acids from a Simulated Primitive Atmosphere

It has been suggested that thermal energy was readily available for the prebiotic synthesis of organic compounds as evidenced by lava remnants from earlier volcanic activity and by the present widespread occurrence of regions of elevated temperatures on the Earth^{1,2}. In previous chemical evolution thermal experiments, the predominant amino acids reported were those commonly found in present-day protein^{1,3,4}. It was implied from these results that thermal energy may have played an important part in the prebiotic synthesis of protein amino acids. It would certainly be significant if thermal energy could produce only these amino acids and not the unusual amino acids of the type identified in simulated primitive atmosphere

discharge experiments^{5,6} and in meteorite extracts^{7,8}, a presumed extraterrestrial abiotic synthesis. Also, previous studies have relied on the identification of amino acids by ion exchange chromatography^{1,4} (amino acid analyser) and electrophoresis³, which is not sufficient for the unequivocal identification of an amino acid, particularly when materials other than the common amino acids may be present^{5,9}. For these reasons we have applied the recently developed techniques of gas chromatography (GC) and gas chromatography combined with mass spectrometry (GC-MS) for identification of the compounds produced by thermal synthesis.

The synthesis was carried out by passing methane (120 ml min⁻¹), which was bubbled through water at 80° C, and ammonia (60 ml min⁻¹) through a 'Vycor' tube containing quartz sand. Reactions were performed with the tube at various temperatures between 900° C and 1,060° C while controls were held at ambient temperature. The gases from a run of 2–3 h were collected in 3 N aqueous ammonia (maintained in an ice bath) and then subjected to a temperature of 75° C for 35 h. The solution was evaporated under reduced pressure and the residue was dissolved in 3 N HCl and refluxed for 5 h. The hydrolysate was divided into two aliquots; one was assayed on a Beckman automatic amino acid analyser, the other treated with *n*-butanol-HCl and trifluoroacetic anhydride to convert amino acids to the *N*-trifluoroacetyl-*n*-butyl esters¹⁰ for analysis by GC and GC-MS. Procedural blanks showed no amino acids.

Six amino acids (α -alanine, glycine, β -amino-*n*-butyric acid, β -alanine, *N*-methyl- β -alanine and aspartic acid) and one dicarboxylic acid (succinic acid) were found in the high temperature (1,060° C) run (Fig. 1), and their identities confirmed by comparison of their mass spectra with those of standard compounds^{9,11}. The number of amino acids produced in this experiment is significantly less than the number reported before^{1,3,4}. Also, in contrast to the predominance of the protein amino acids found in earlier studies, only three (glycine, α -alanine and aspartic acid) were identified. Our work seems to be unique in one other aspect. Reviewing the chemical

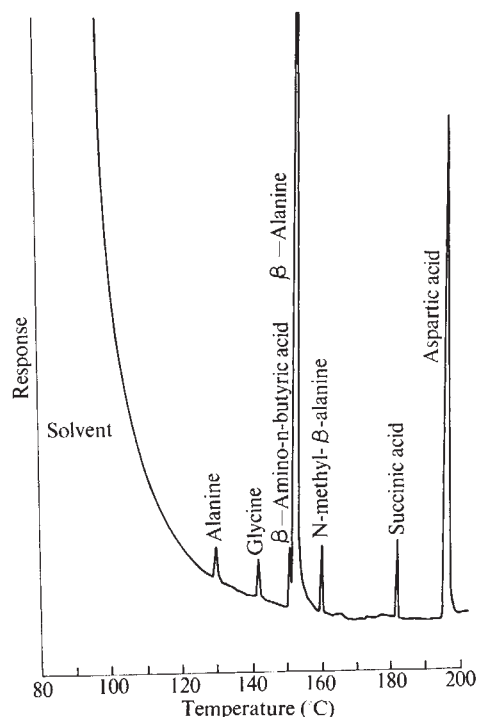


Fig. 1 Gas chromatogram of *N*-trifluoroacetyl-*n*-butyl esters of amino acids from the thermal synthesis at 1,060° C. Gas chromatography: 0.02 inch $\times$ 50 foot capillary column; OV17 coating; flame ionization detector; isothermal at 80° C for 4 min, then programmed to 200° C at 8° C min⁻¹.

Table 1 Compounds Produced by Thermal Synthesis

Compound	1,060° C (mol %)*	980° C (mol %)*	930° C (mol %)*
α -Alanine	1	12	4
Glycine	1	59	96
β -Alanine	90	28	—
<i>N</i> -Methyl- β -alanine	1.5	1	—
Succinic acid	1.5	—	—
β -Amino- <i>n</i> -butyric acid	1	—	—
Aspartic acid	3	—	—

* Yields were determined by amino acid analyser and gas chromatographic response.

evolution literature¹², Lemmon pointed out that in all simulated prebiotic experiments the yield of α -alanine was always higher than the yield of β -alanine. In our high temperature experiment, however, the yield of β -alanine (90 mol %) was approximately two orders of magnitude greater than the yield of α -alanine (1 mol %), and the yield of all compounds with β -separation of functional groups was much greater than those with α -separation of functional groups (Table 1).

The reactions and interactions of a series of intermediates (aminoacetonitrile, α -aminopropionitrile, hydrogen cyanide, methylamine, acrylonitrile, cyanoacetylene and crotononitrile) can be postulated to account for the products observed. Glycine and alanine would be the expected hydrolysis products of the corresponding nitriles (aminoacetonitrile and α -aminopropionitrile). β -Alanine has been identified in discharge experiments¹³ and was postulated to be the hydrolysis product of β -aminopropionitrile, produced by Michael addition of ammonia to acrylonitrile. Also, acrylonitrile was predicted to produce succinic acid and *N*-methyl- β -alanine from hydrolysis of the nitrile intermediates originating from Michael addition of hydrogen cyanide and methylamine. Succinic acid was identified, but *N*-methyl- β -alanine, which is insensitive to the ninhydrin reaction commonly used in amino acid analysers, was not detected. In our synthesis at 1,060° C, we identified β -alanine, succinic acid and *N*-methyl- β -alanine, a finding consistent with the addition of ammonia, hydrogen cyanide and methylamine to acrylonitrile. The sequential addition of ammonia and hydrogen cyanide to cyanoacetylene is proposed as a route to a nitrile intermediate which is converted to aspartic acid after hydrolysis. Aspartic acid has been previously observed to be the product of such a reaction sequence¹⁴. The final compound identified, β -amino-*n*-butyric acid, would be the expected product obtained by hydrolysis of the nitrile intermediate resulting from addition of ammonia to crotononitrile.

The amino acids produced in lower temperature experiments are included in Table 1. At 980° C the concentration of β -alanine still exceeded the concentration of α -alanine; however, the α -amino acids were more abundant than the β -amino acids. Also, aspartic acid, β -amino-*n*-butyric acid and succinic acid were absent, indicating lack of the proposed intermediates hydrogen cyanide and crotononitrile. At this temperature, the ratio of glycine to α -alanine was approximately 5 to 1, whereas at 1,060° C the ratio was 1 to 1. At 930° C glycine and alanine were the only principal products, with the ratio of glycine to alanine nearly 20 to 1. These results suggest a temperature dependence for the proposed intermediates.

The yield from these experiments is low, typically 6–70 μ mol of carbon incorporated into amino acids for each mole of methane used. This represents a yield of at best 0.007%, which is small compared with the 1.9% recently reported for a discharge experiment⁸.

The unusual nature of the results presented here (predominance of β -amino acids at high temperature, limited number of amino acids produced, and low yield) presents problems in the interpretation of their relevance to chemical evolution. If these results are representative of what may have taken place on the primitive Earth, then the small yields of the thermal

experiments are discouraging, particularly when compared with the discharge experiment which is postulated to have been a more abundant energy source for synthesis¹⁵. One way to circumvent this problem is to invoke a localized high concentration of thermally produced amino acids near a heat source such as a volcano. As vent temperatures are 1,050°–1,150° C¹⁶, however, the products of such a synthesis would contain predominantly β -amino acids by analogy with the 1,060° C experiment. The formation of a primitive organism in an environment requiring the utilization of β -amino acids, followed by evolution into a modern organism that utilizes α -amino acids, is unattractive.

An alternative interpretation of our results is that they are not representative of what may have happened on the primitive Earth. If it is assumed that thermal synthesis was important for the prebiotic synthesis of amino acids, then future laboratory experiments should be designed to overcome the problems we encountered.

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Evidence for the Possible Immunogenicity of Δ^9 -Tetrahydrocannabinol (THC) in Rodents

CHRONIC marihuana intoxication is accompanied by a marked pharmacological tolerance in all animal species^{1–3}, for example the necessity to increase dosage to obtain the effect of initial dosage. A similar degree of tolerance also develops to the effects of Δ^9 -tetrahydrocannabinol (THC), the chief psychoactive substance of marihuana^{4,5}. The time period required for the development of tolerance to THC in rodents⁵ or dogs⁶ is of the order of 5 to 9 d, which is similar to that required for the development of a cellular immune response. It was also shown by Agurell⁷ that 3 d after the intravenous injection of tritiated Δ^9 -THC there was a very high specific activity in the spleen.

These observations led us to suggest⁸ that tolerance development to marihuana and to Δ^9 -THC might be mediated in part by the immune system, although no circulating antibodies to THC have ever been isolated.

Two series of experiments were performed to test this hypothesis. In the first we studied the effect of an immunosuppressant, azathioprine, on the development of tolerance to the hypotensive effects of THC in the spontaneously hypertensive rat (SHR). In the second we studied the effect of THC on blast transformation of lymphocytes sampled from mice sensitized to THC.

In the first series, three groups of twelve SHR rats weighing 234 ± 2 g were studied. The two control groups were given daily either azathioprine (4 mg kg^{-1} i.p.) or THC by gastric tube (10 mg kg^{-1} in sesame oil). The third group was given 4 mg kg^{-1} azathioprine and 10 mg kg^{-1} THC. Treatment with azathioprine was started 2 d before administration of THC. Blood pressure was measured each day by tail plethysmography 24 h after administration of the drug.

Administration of azathioprine alone does not alter blood pressure. THC administration produces a significant hypotension for only 6 d, as previously reported⁵. Pharmacological tolerance to this effect then follows. By contrast, the animals treated with THC and azathioprine do not develop tolerance to the hypotensive effects of THC during the experimental period of 13 d (Fig. 1). One may ask whether the immuno-

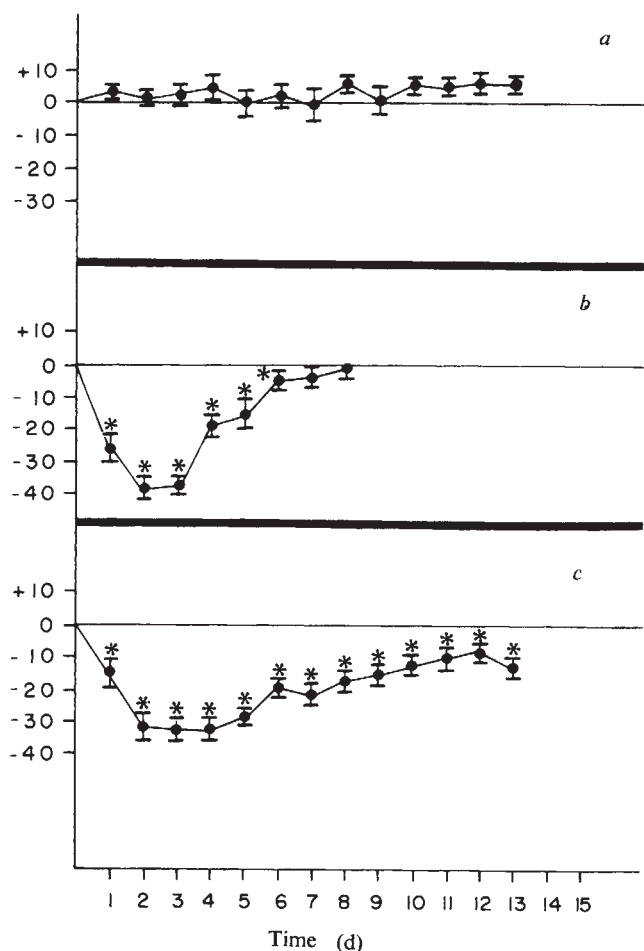


Fig. 1 Effects of an immunosuppressant, azathioprine, on the development of the hypotensive effects of Δ^9 -THC in the spontaneously hypertensive rat (SHR). Twelve animals were used in each group. a, Azathioprine, $4 \text{ mg kg}^{-1} \text{ d}^{-1}$; mean control blood pressure 195 ± 3 mm Hg; b, THC, $10 \text{ mg kg}^{-1} \text{ d}^{-1}$; mean control blood pressure 199 ± 3 mm Hg; c, azathioprine, $4 \text{ mg kg}^{-1} \text{ d}^{-1}$ + THC, $10 \text{ mg kg}^{-1} \text{ d}^{-1}$.

*Significantly different from control blood pressure changes ($P < 0.05$).

suppressant therapy inhibited the development of pharmacological tolerance to THC.

In the second series, three groups of five male mice of the CD1 strain were used. The two experimental groups were given, at one week intervals, two intravenous injections of THC in alcohol (5 mg kg⁻¹) and one intraperitoneal injection (10 mg kg⁻¹) in Freund adjuvant. The control group was given similar injections of the same volume of vehicle. All animals were killed 24 to 48 h after the last injection.

The spleens of five animals were pooled and sectioned in fine fragments in culture medium RPMI 640, pH 7.2. The cellular suspension thus obtained was filtered on a grid of mesh 60, washed and adjusted so that the cell concentration was 3.5×10^6 cells ml⁻¹.

Because of the toxicity of alcohol to lymphocytes, THC was added to the cell culture after preparation as follows⁹. The alcohol of the THC solution was evaporated by bubbling nitrogen. The resinous residue was then resuspended by magnetic stirring for 12 h at 4° C in a solution of foetal calf serum containing 50 mg of bovine serum albumin (BSA) ml⁻¹. (In such a preparation, 85% of the THC is recovered in the BSA solution⁹.) THC in BSA and foetal calf serum was added to the samples so that the final concentration of THC was 5 mg l⁻¹ (1.6×10^{-5} M). THC vehicle was added to the control samples, and in one group of controls phytohaemagglutinin P (PHA P, Difco), suspended in sterile water, was added at 10 µl per tube. After 48 h of incubation at 37° C, 2.5 µCi of ³H-thymidine with a specific activity of 1.9 Ci mmol⁻¹ was added to the samples for the final 24 h of culture.

At 72 h the tubes were placed on ice. After two washings, in RPMI, cells were spread on glass slides, fixed in methanol, and dipped in an Ilford emulsion. After 30 h exposure they were developed and stained with Wright Giemsa; those cells which had incorporated ³H-thymidine were counted. The results are given as the percentage of cells in the population scored as lymphoblasts (1,000 to 1,500 cells were counted in each experiment). Adler *et al.*¹⁰ have reported that the time course of lymphoblastic transformation appreciated by morphological criteria as described by Chessin *et al.*¹¹ was paralleled roughly by incorporation of ³H-thymidine, in both stimulated and unstimulated cells. The autoradiographic technique which we used is more accurate as only cells which have incorporated ³H-thymidine are counted.

Table 1 indicates that THC, unlike other substances of vegetable origin such as phytohaemagglutinin (PHA), does not stimulate mitosis of lymphocytes in culture. Furthermore, the lymphocytes of mice treated with THC and incubated in the presence of the drug incorporate ³H-thymidine at a faster rate, as indicated by the increased blast transformation. The specific blastogenic activity of THC is similar to that induced by other antigenic substances. It is observed only on spleen cells sampled from animals sensitized to the same antigen as the one which is introduced into the culture.

The blast transformation we have observed with THC is similar in extent to that observed with other antigens, but is much less than that stimulated by PHA. These experiments add further weight to the hypothesis we suggested previously⁸. The inhibition of tolerance development in animals treated with

azathioprine could be related to an inhibition of the immune response.

That a small molecule like THC (molecular weight 314.5) could induce a cellular immune response usually produced by macromolecules seems at first unlikely. But THC, which is insoluble in water, is transported in the plasma, 90% bound to protein¹². It is conceivable that THC might constitute a hapten *in vivo* which could trigger an immune response, as indicated by blast transformation. There is a similar immune response towards morphine in mice¹³.

If a similar immune response to THC occurs in man, it could account for the eosinophilia¹⁴ and the allergic responses which have been observed among cannabis users^{15,16}. It could also account for the extreme variability of individual reactions reported after usage of this drug¹⁷.

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Production of Antibody to Sheep Red Blood Cells by Human Tonsil Cells *in vitro*

Two types of lymphocyte cooperate in initiating the humoral response to antigen: the B cell is the progenitor of antibody-forming cells and the T cell acts in a helper capacity. The activity of a third cell, the macrophage, is also required. Although the functions of B and T cells are antigen-specific, that of the macrophage is not. Most knowledge about the kinetics and distribution of the two types of lymphocytes, their specificity, and their susceptibility to inhibition or stimulation, has been established by experiments with mice. Progress has been facili-

Table 1 Percentage Blast Transformation of CD1 Spleen Cells Observed after ³H-Thymidine Incorporation. Cells from Five Pooled Spleens were Used in each Experiment

	Control mice	Mice treated with THC	
		Exp. I	Exp. II
Lymphocytes in culture without THC	4.7%	7.2%	6.8%
Lymphocytes in culture with THC	4.3%	13.5%	13.1%
Lymphocytes in culture with PHA	37.8%	—	—

Table 1 Lack of Production of Antibody to SRBC by Cultures of Human Blood Lymphocytes and Tonsil Cells from Individual Donors

Cultured cells	PFC* on day 5	
	with SRBC†	without SRBC
Blood	0	0
Blood	0	0
Blood	0	0
Tonsil	42	68
Tonsil	3	6
Tonsil	0	0

* Plaque-forming cells per culture.

† 3×10^5 SRBC per culture.

tated in particular by the development of methods that measure the response of mouse spleen cells to antigen *in vitro*; we report here some experiments with human lymphocytes from blood and tonsils, the two sources of such cells most readily available.

We used the culture method described by Mishell and Dutton¹ and the ficoll-hypaque method for purifying blood lymphocytes described by Boyum². The tonsils were cut in half with a hot scalpel blade, and cells were obtained from the cut surface by mechanical dissociation.

Culture with sheep red blood cells (SRBC) did not induce blood lymphocytes or tonsil cells from individual donors to produce antibody to SRBC (Table 1). This was taken to indicate that one or more of the cellular components (T cells, B cells or macrophages) did not function properly or were not present in adequate numbers. To compensate for a possible lack of macrophages in the tonsil cell preparations, we added increasing numbers of blood leukocytes (known to be rich in phagocytic cells) to the tonsil cells. Table 2 shows that these mixed cultures produced antibody to SRBC in the presence of SRBC. Because the blood cells and tonsil cells used in these experiments came from different donors, the results could be interpreted in two ways: (1) the addition of blood monocytes did in fact compensate for a deficiency in phagocytes of the tonsil preparation, or (2) the response was a consequence of stimulation by foreign alloantigens, which can amplify or replace the specific helper function of T cells in the mouse^{3,4}.

Table 2 Production of Antibody to SRBC by Mixed Cultures of Tonsil Cells and Blood Lymphocytes

Tonsil cells	Blood lymphocytes	Day 4	PFC Day 5	Day 6
5×10^6	—	13	22	25
—	5×10^6	—	3	16
5×10^6	10^6	105	286	380
5×10^6	3×10^5	77	122	175
5×10^6	10^5	18	76	41
5×10^6	3×10^4	6	17	5

Subsequent experiments favoured the latter explanation. (a) Tonsil cells that did not produce antibody to SRBC, when cultured individually, did so in mixed culture (Table 3), showing that human tonsils do contain B cells able to respond to SRBC; the same cannot be said of blood lymphocytes because they did not produce antibody to SRBC even in mixed cultures. (b) The positive response of mixed tonsil cultures, differing only in alloantigens, as opposed to individual cultures, suggests that tonsils contain sufficient macrophages. This is also indicated by the fact that neither mouse macrophages nor 2-mercaptoethanol⁵ induced antibody production to SRBC in individual tonsil cultures.

Thus T cell deficiency rather than macrophage deficiency seems to be the limiting element in the tonsil cell population. Because of a recent report that bacterial lipopolysaccharides can substitute for T cell function in mice⁶ we then investigated the effect of *E. coli* endotoxin on the production of antibody to SRBC by human tonsil cells. As Table 4 shows, the addition of *E. coli* lipopolysaccharide to cultures of individual tonsils induced the production of antibody to SRBC, and the response

Table 3 Production of Antibody to SRBC by Mixed Tonsil Cell Cultures

Source of tonsil cells	PFC on day 5	
	with SRBC†	without SRBC
A*	32	30
B	6	8
C	14	18
A+B	170	80
A+C	88	6
B+C	200	48
D	8	4
E	76	18
F	104	100
D+E	100	44
D+F	600	208
E+F	520	144

* 10^7 cells per culture; 5×10^6 cells from either source in mixed cultures. The letters designate tonsils from different subjects.† 3×10^5 per culture.

was of the same magnitude as that seen in mixed cultures. We could also elicit the production of hapten-specific antibody to 2,4,6-trinitrophenol, conjugated with SRBC, by adding endotoxin to cultures of tonsil cells. The latter finding offers a means of investigating the function of T and B cells independently, using the method developed for the mouse⁷. So far, our experiments have shown differences in responsiveness of individual tonsils depending, for example, on the age of the donor and on the size of the tonsil.

Table 4 Effect of *E. coli* Lipopolysaccharide on the Production of Antibody to SRBC by Cultured Tonsil Cells

Source of tonsil cells*	<i>E. coli</i> lipopolysaccharide†	PFC on day 4	
		with SRBC‡	without SRBC
G	—	116	8
	+	590	28
H	—	102	100
	+	1,260	280
I	—	65	110
	+	440	170
K	—	6	6
	+	97	26
L	—	0	0
	+	10	0
M	—	24	12
	+	52	14

* 10^7 per culture; the letters designate tonsils from different subjects.

† 0.030 mg per culture.

‡ 3×10^5 per culture.

The background of the plaque-forming cells in mixed tonsil cultures, and in cultures of individual tonsils to which *E. coli* lipopolysaccharide had been added, is not at present explained. It may indicate pre-existing sensitization to cross-reacting sheep antigens, red cell antigens or bacterial antigens or, in the case of *E. coli* lipopolysaccharide, cross-reactivity between this material and SRBC antigens.

In vitro studies of this sort may lead (as they have done in the mouse) to better understanding of factors that govern the immune response in man, and may suggest ways of manipulating them for the purpose of correcting immunological defects.

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Basis of the Defect in α -1-Antitrypsin Deficiency

DEFICIENCY of the serum protein α -1-antitrypsin is an important cause of obstructive lung disease in adults and of hepatitis in children. The deficiency state can be considered as one variant form of α -1-antitrypsin but several other variants are also known, occurring with a frequency of approximately three per hundred in Northern Europeans¹. For the most part these are simple electrophoretic variants which are functionally normal and explicable as simple amino acid substitutions. The proportion of the variant form, and family studies, strongly support a single autosomal locus for α -1-antitrypsin.

There are, however, some perplexing observations. First, whereas electrophoresis of α -1-antitrypsin in agar gel (pH 8.6) gives a single band, electrophoresis in borate starch gel at pH 4.9 gives a complex multiband pattern (Fig. 3a). This pattern has been well studied and is used by Fagerhol and Laurell² in their Pi system, which provides a nomenclature for classifying the variant genes (for example *M* for the normal gene, *Z* for the deficient gene). It has been difficult, however, to explain fully these complex patterns in terms of a single locus and they could be interpreted as evidence of considerable genetic heterogeneity. Another perplexing feature is seen in the homozygous deficiency state (*ZZ*) in that the deficiency is never complete, some 10% of the α -1-antitrypsin always being present in the serum. Furthermore, this remnant α -1-antitrypsin has a distinctly slower mobility than normal on agar gel electrophoresis. In acid starch gel electrophoresis the *ZZ* pattern (Fig. 3b) differs even more markedly in both distribu-

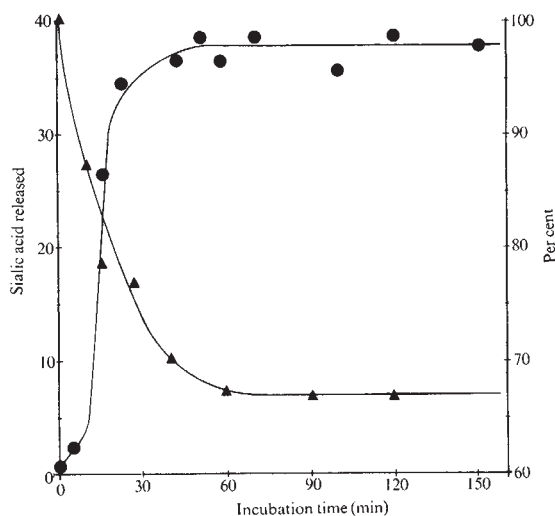


Fig. 1 Incubation of α -1-antitrypsin with neuraminidase gave a decrease in electrophoretic mobility concomitant with the release of sialic acid (N-acetylneuraminic acid). The mobility change ($\blacktriangle$; % of normal) was determined using immunoelectrophoresis in agar gel (see Fig. 2). The sialic acid release ($\bullet$) is given in $\mu\text{mol} \times 27 \times 10^3$ per ml of extract.

- (i) 120 min
(ii) Extract
(iii) MM
(iv) ZZ
(v) 5 min
(vi) 10 min

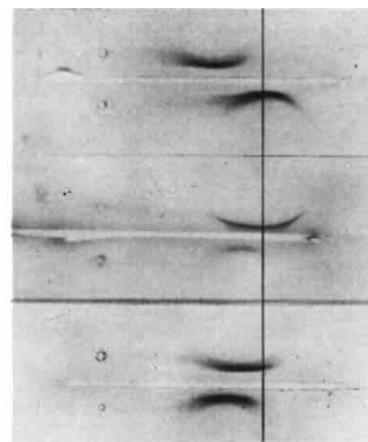


Fig. 2 Immunoelectrophoresis (agar, pH 8.6) against specific α -1-antitrypsin antiserum. The prepared extract (ii) shows the normal *MM* mobility as indicated by the drawn line. Incubation of the extract with neuraminidase shows a progressive decrease in mobility (V, VI, I), the 5 min incubation sample (V) having a 10% decrease in mobility equivalent to that seen in the deficiency *ZZ* state (IV).

tion and mobility from the *MM* pattern. Together these findings make it difficult to explain the deficiency state on the basis of a simple genetic lesion.

We believe that the electrophoretic findings can be explained in terms of the carbohydrate moiety of α -1-antitrypsin and that a lesion in the structure of this would explain both electrophoretic and morphological findings in the homozygous deficiency state.

The electrophoretic heterogeneity of α -1-antitrypsin is not contradictory of a single locus and has been similarly observed with other glycoproteins (for example porcine RNase). It is possible that the banding represents microheterogeneity of the carbohydrate though the requirement of borate in the α -1-antitrypsin system suggests a complexing that may vary with ordered structure. Supporting evidence that the banding reflects variations in ordered structure is our finding that addition of 6 M urea to the borate acid starch gel system results in a single band of α -1-antitrypsin.

We also found that the appearance of the *ZZ* antitrypsin could be duplicated by partial removal of sialic acid (N-acetylneuraminic acid) from the normal α -1-antitrypsin. Human α -1-antitrypsin was separated from other glycoproteins by preparative electrophoresis in polyvinyl chloride/acetate. The eluted and concentrated material was then subjected to timed digestion with neuraminidase (Type VI, Sigma) and measurements made of sialic acid release⁴ and of changes in the electrophoretic mobility of the α -1-antitrypsin in agar gel at pH 8.6. As shown in Fig. 1 the mobility decreased concomitantly with the release of sialic acid, the final mobility being approximately 70% of that of the normal. As Fig. 2 shows, the *ZZ* antitrypsin has a 10% decrease in mobility equal to that after incubation for 5 min in the system used and equivalent to one third the decrease of the complete reaction. Incubation of whole human serum was then performed using neuraminidase concentrations calculated to give appropriate partial digestion. Electrophoresis confirmed that there was again a slowing of α -1-antitrypsin equivalent to the *ZZ* form. More significantly, electrophoresis in acid starch gel gave a striking reproduction of the *ZZ* pattern both in the mobility and proportion of peaks (Fig. 3c). Both the *ZZ* antitrypsin, and the normal treated with neuraminidase, retain their antitryptic activity⁵.

Thus the changed appearance of α -1-antitrypsin in the deficiency state can be explained by a partial loss of sialic acid. This fits in well, together with other observed changes which have been reviewed by Sharp⁶ to give a coherent hypothesis for the disease process. The carbohydrate moiety of glycoproteins

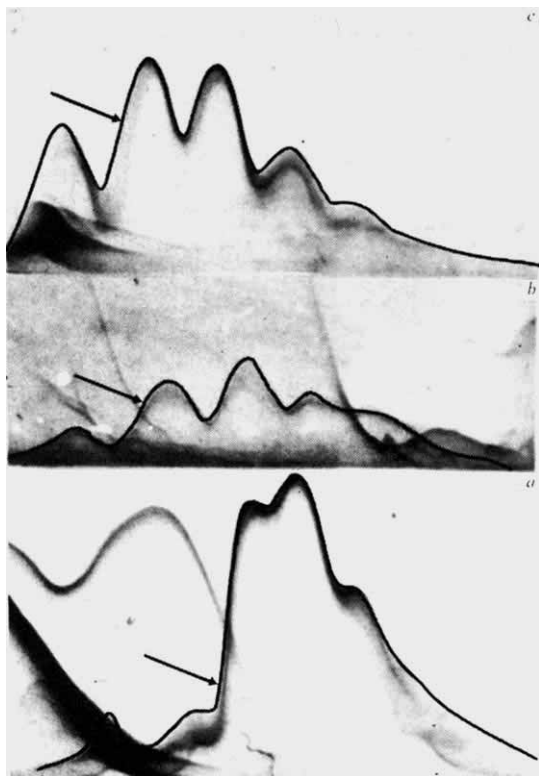


Fig. 3 Two dimensional immunoelectrophoresis of serum. First dimension starch gel pH 4.9, second dimension agar containing polyvalent antisera. The three samples have been run under the same conditions and aligned in terms of the buffer front and origin. The α -1-antitrypsin line is arrowed and outlined. (a), Normal (MM) pattern; (b), concentrated homozygous deficient (ZZ) serum showing characteristically diminished peaks of slower mobility; (c), normal serum incubated with neuraminidase. The ZZ distribution is closely reproduced.

is almost certainly of importance for their active transport across the cell membrane. We propose that in the deficiency state there is incomplete addition of sialic acid to the molecule, which can be expected to accumulate intracellularly with only small amounts escaping by passive diffusion. In fact, α -1-antitrypsin in the deficiency state accumulates in large amounts at the site of synthesis in the liver cells. In particular it is associated with the rough endoplasmic reticulum which is believed to be a site of synthesis of the carbohydrate portion of glycoproteins.

The accumulation within the liver cell renders it particularly susceptible to hepatitis and is thought to be the major factor in the development of the associated childhood cirrhosis. Small amounts of antitrypsin may be released by passive diffusion to give the circulating ZZ antitrypsin but overall levels are presumably insufficient to protect lung tissue from proteolysis.

It is therefore our view that the deficiency state for α -1-antitrypsin can be explained on the basis of a single locus of origin, the defect being in the synthesis of the carbohydrate moiety resulting in defective release from liver cells.

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Glycine N-Methyltransferase is a Regulatory Enzyme which Increases in Ageing Animals

TRANSFER RNAs are modified extensively after their primary synthesis is completed. Such modifications, which are necessary for the proper functioning of tRNA in protein synthesis¹⁻³ and transcriptional control⁴, are achieved by enzymes which include the tRNA methyltransferases⁵. In certain adult organs the tRNA methyltransferases are under the influence of a competing methylating enzyme, glycine N-methyltransferase⁶. This enzyme system, which is present in adult liver, kidney and pancreas (but not in the corresponding foetal organs or in tumour tissues), not only competes for the methyl donor, S-adenosylmethionine, but is much less sensitive to inhibition by the product of the reaction, S-adenosylhomocysteine, than are the tRNA methyltransferases. Thus, there is a dual mechanism of control.

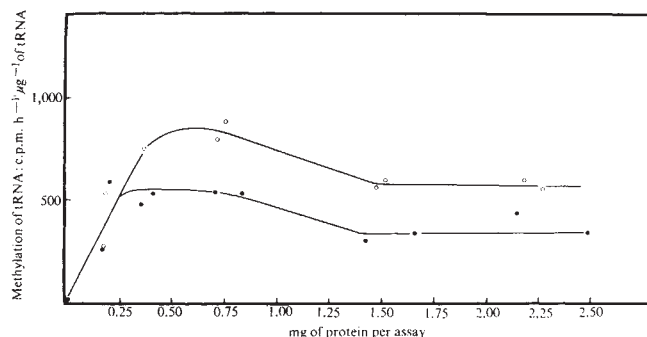


Fig. 1 Transfer RNA methyltransferase capacity of adult rat liver. Extracts prepared as for glycine N-methyltransferase assay⁶ were assayed for tRNA methylase capacity¹³ using 1 μ g of *E. coli* B tRNA per assay as substrate. Protein was assayed according to Lowry *et al.*¹². Male Sprague-Dawley rats were used. ○, Livers from two 3-month-old male rats; ●, from two 12-month-old male rats.

We report here that glycine methyltransferase activity is increased significantly in the organs of rodents during post-natal development and ageing. This finding may imply a restricted activity of the tRNA methyltransferases in ageing animals with a possible consequent deficiency of modification of the tRNA populations.

The increase in glycine N-methyltransferase activity is not merely a property of the transition from foetal to adult organism. As Table 1 shows, the specific activity of the glycine N-methyltransferase in rat liver increased 30% during the period between 3 and 12 months, while in rat kidney the increase was 40%.

The effect of these increases in glycine N-methyltransferase activity on the methyltransferase capacity of 3 months (post-pubertal) and 12 months (mature) rat liver and kidney *in vitro* can be seen in Figs. 1 and 2. These assays are performed using crude, undialysed 100,000g supernatant extracts of the organs. Thus, glycine is present as well as glycine N-methyltransferase. At low protein concentrations, where the competing enzyme system is diluted out, the specific activities of the tRNA

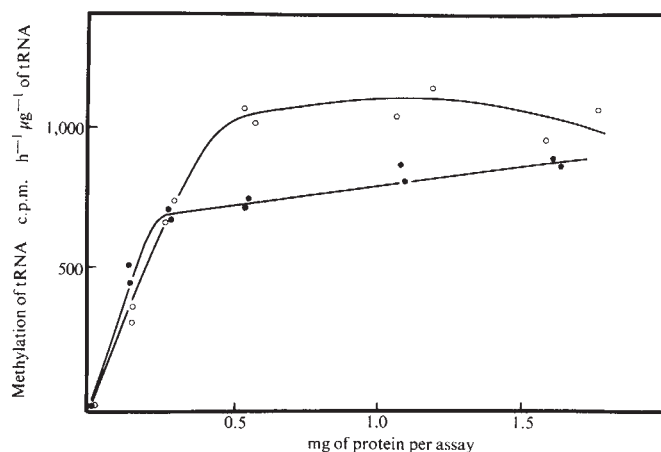
Table 1 Specific Activity of Glycine in N-Methyltransferase in Liver and Kidney of Post-pubertal and Mature Rats

Organ	Post-pubertal 3 Months	Mature 12 Months
Liver	22.6	31.0
Kidney	3.6	5.8

Specific activity is expressed as nmol sarcosine formed per mg protein per 30 min. Extracts were prepared and assayed for activity according to Kerr⁶, except that $^{14}\text{CH}_3\text{-S-adenosylmethionine}$ (specific activity 53 Ci mol⁻¹) was present at a concentration of 2×10^{-5} M. Protein was assayed according to Lowry *et al.*¹² Rats were male Sprague-Dawley animals.

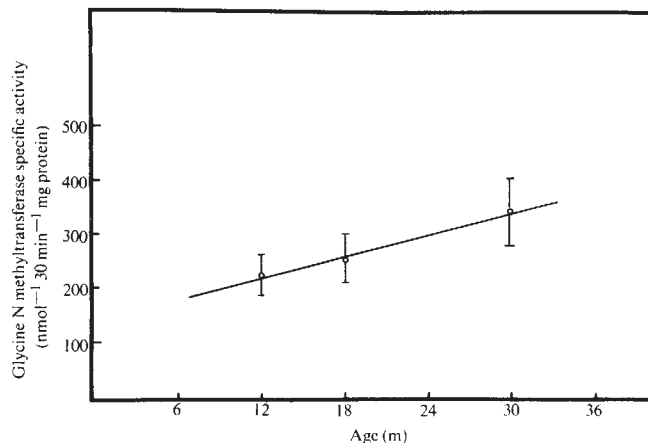
Age	Body weight		Range (g)
	No. of animals	Average (g)	
3 months	4	267	235–309
12 months	8	429	391–471

methyltransferases from 3 and 12-month-old rats seem to be the same. At the higher protein concentrations required to reach saturation capacity, however (the maximum number of methyl groups which the enzymes from a given source can introduce into a unit amount of heterologous tRNA), there is a marked difference in the saturation levels reached by the tRNA methyltransferases of organs from 3 and 12-month-old rats. The total capacity for tRNA methylation is lowered in the older animals by about 35%.

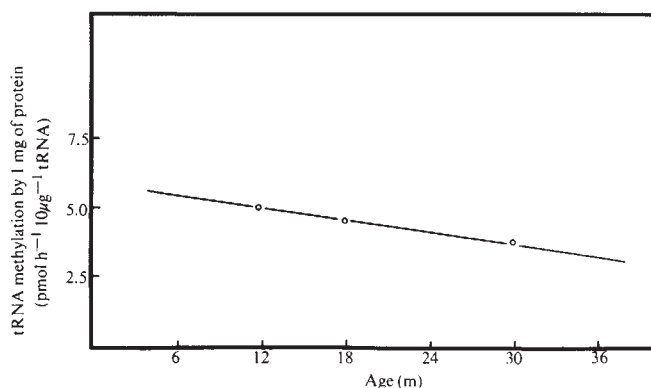
**Fig. 2** Transfer RNA methyltransferase capacity of adult rat kidney. Experimental details were as for Fig. 1. ○, Kidneys from two 3-month-old male rats; ●, from two 12-month-old male rats.

A similar increase in glycine N-methyltransferase specific activity was observed in the livers of C57Bl/6J male mice. Between 12 months (mature) and 30 months (senescent), which is the average life span⁷, the specific activity of the enzyme undergoes a linear increase (Fig. 3). The specific activity of tRNA methyltransferase of mouse livers is also diminished with age (Fig. 4).

These measurements demonstrate that glycine N-methyltransferase increases during ageing as well as during maturation. If the concomitant decrease in activity of tRNA methyltransferase *in vitro* is an accurate reflexion of the activity of these enzymes *in vivo* then the tRNA of ageing animals may become progressively hypomethylated with a possible decrease in functional fidelity. Changes in tRNAs and their modification have been reported in two different ageing biological systems.

**Fig. 3** Glycine N-methyltransferase activity of adult C57Bl/6J male mouse liver. Livers were frozen in dry ice immediately after death. They were subsequently held at -80°C until use. Preparations and assays were as described in Table 1 except that S-adenosylmethionine was used at 5×10^{-5} M in all reactions. Each mean is from two determinations on each of five animals. Careful husbandry procedures and observations at necropsy^{7,16} eliminated any mice with gross pathological lesions, particularly the reticulum cell sarcoma which is characteristic of senescence in this strain⁷. ○, Mean (livers) from five mice; I, standard deviation.

Differences in tRNA from aged compared with young mosquitoes have been reported⁸, and in the apical part of the wheat-leaf, which has stopped growing, there is a tRNA^{Phe} which is improperly modified and consequently its attachment to ribosomes is impaired⁹. A hypothesis of random accumulation of errors in proteins synthesized, as being instrumental in the ageing process, has been previously proposed¹⁰ and the accumulation of altered proteins in ageing human fibroblasts in tissue culture has been recently demonstrated¹¹.

**Fig. 4** Transfer RNA methyltransferase activity in C57Bl/6J male mouse livers. Liver treatment was the same as for Fig. 3. The tRNA methyltransferase activity assay was as for Fig. 1 except with 10 μg of *E. coli* B tRNA per assay. Protein was assayed according to Lowry *et al.*¹².

Increased glycine N-methyltransferase activity and putatively consequent hypomethylation during post-natal development and ageing could result in the appearance of novel isoaccepting tRNA species with ensuing effects on protein synthesis. A novel serine tRNA is found, for example, in the uterus of ovariectomized rats, whose tRNA methyltransferase activity is diminished¹⁴. Because of the sensitivity of tRNA methylation to hormones, it is possible that the increased glycine N-methyltransferase activity represents the response of the liver and kidney to changes in hormones related to age, as has

been shown for certain other age-related changes of enzyme activities^{15,16}.

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Prenatal Hypothyroidism and Brain Composition in a Primate

THE neonatal rat has been used almost exclusively as a convenient model for elucidating the effects of thyroid hormone or thyroid ablation on the developing central nervous system¹⁻⁴.

Although valuable information has been obtained, the growth and development of rats differs from that of primates^{5,6}. Recent studies⁷⁻¹⁰ have shown that the monkey (*Macaca mulatta*) is a more suitable model for the evaluation of experimental situations applicable to the human foetus. Six pregnant rhesus monkeys were injected with 2 mCi Na¹³¹I kg⁻¹ intravenously at 71-88 d of gestation. Cesarean section was performed on these and an equal number of untreated pregnant females at 150 d (full term gestation is 165 ± 4 d).

Foetal and maternal plasma thyroid hormone levels were indicative of hypothyroidism in the ¹³¹I-treated animals (Table 1) although the mothers showed no apparent clinical symptoms during pregnancy. The treated foetuses weighed somewhat less than expected for gestational age and showed reduced respiratory movements and cretinoid facies. The thyroid gland was completely absent. The weight of the whole brain (including its parts) was within normal limits but the pituitary was heavier than normal. Details on physical growth and skeletal maturation including a discussion of placental thyroid hormone transfer have been published elsewhere¹¹.

Table 1 Thyroid Hormone in Foetal and Maternal Plasma

	Maternal	Foetal
Protein-bound iodine (µg%)		
Control	7.2 ± 1.2	7.3 ± 0.5
¹³¹ I	4.6 ± 1.4	4.7 ± 0.4
Thyroxine (µg%)		
Control	9.6 ± 2.3	7.2 ± 2.1
¹³¹ I	2.3 ± 0.9	0.6 ± 0.8

Cerebrum and cerebellum were analysed separately for DNA¹², RNA¹³, protein¹⁴, Na⁺ + K⁺-ATPase^{15,16} and carbonic anhydrase activity¹⁷, cholesterol^{18,19}, lipid N-acetylneuraminic acid (lipid NANA)^{18,20} and chloride²¹.

Water was determined by desiccation and partitioned into apparent extracellular and intracellular compartments assuming that the chloride space is a valid measure in this situation. Stereological measurement of the extracellular space of developing rat cortex following a freeze-substitution technique²², which agrees with chloride space determinations in similar samples²³, adds credence to this approach.

The data are expressed in terms of total amount and are presented as percentages of the normal control values. In Fig. 1, the cerebrum had essentially the same mean weight, water and DNA content (cell population) as the normal controls. Total protein and non-protein dry solids (lipid, polysaccharides and mineral) were significantly reduced. The non-chloride space (an index of neuronal volume)²³ was significantly reduced while the concomitant increase in chloride space was not significantly different. The cerebellum showed a similar but more marked pattern with highly significant reductions in non-chloride space (21%), protein (23%) and non-protein dry solid (15%).

Fig. 2 documents the changes in RNA, two neuronal components (lipid NANA and total N⁺ + K⁺-ATPase activity) and two extraneuronal components (cholesterol and total carbonic anhydrase activity). Changes in weight, DNA and protein are reproduced for comparison. RNA was significantly reduced in the cerebrum (17%) and cerebellum (19%). Cerebral neuronal components—lipid NANA and Na⁺ + K⁺-ATPase—were deficient by 17% and 34% respectively, and in the cerebellum both these components were reduced 30%. Cerebral cholesterol and carbonic anhydrase activity were reduced 16% and 43% respectively. In the cerebellum a greater reduction of these components was observed. The cholesterol content was reduced 28% and the total carbonic anhydrase activity was reduced 48% by comparison with control values.

Many of the changes described here have been previously

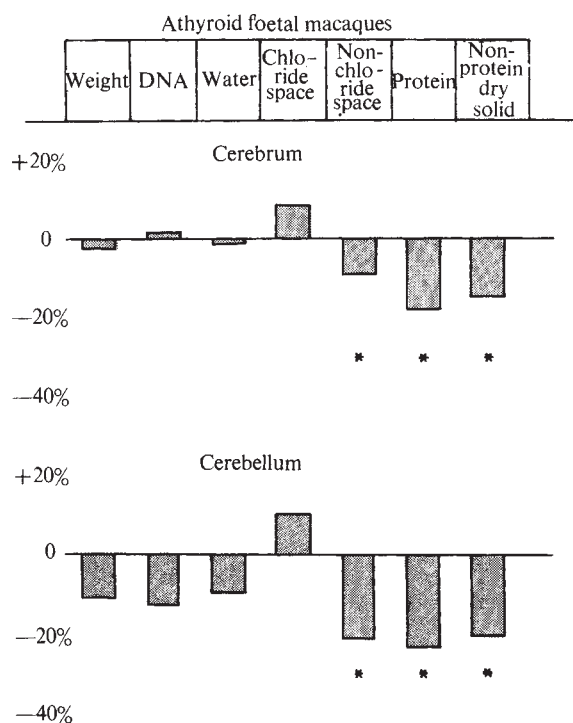


Fig. 1 Changes in the gross composition of the cerebrum and cerebellum of athyroid foetal macaques. Each component is represented as the mean percentage change relative to the control (zero line). Weight and DNA are included for reference. The asterisk indicates statistical significance. $P < 0.01$.

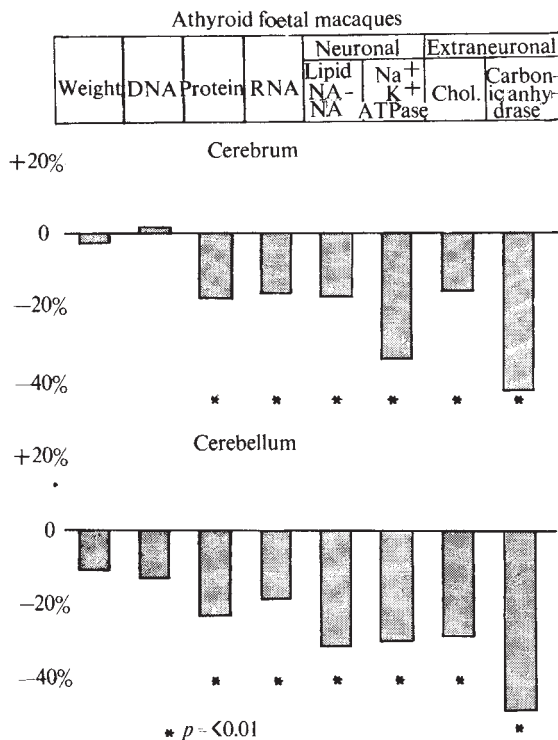


Fig. 2 Changes in the content of RNA, neuronal and extra-neuronal components of the cerebrum and cerebellum of athyroid foetal macaques. Weight, DNA, and protein are illustrated for reference. The data are presented in the same way as in Fig. 1.

observed in young rats receiving thyroid ablation at birth^{1-4,24}. Data describing the chemical composition of the brain in this rat extrauterine model include normal DNA contents with absolute reductions in RNA, protein and cholesterol. Elevation in the percentage of water and changes in chloride concentration have also been described. Reduction in $\text{Na}^+ + \text{K}^+ - \text{ATPase}$ activity has been consistently demonstrated^{25,26}. This depression, when expressed as activity per gramme was greater for cerebral cortex than for the cerebellum. A similar pattern is formed in the foetal macaque brain in the absence of thyroid function. It is emphasized that increase in DNA concentration in the cerebrum and reduction in the cerebral weight, which occur in the neonatal hypothyroid rat, were not observed in this study.

Carbonic anhydrase, an enzyme found exclusively in glia²⁷, is greatly reduced in the brains of our athyroid foetuses. This enzyme has not been determined in the hypothyroid rat brain. Cholinesterase activity (which predominates in glial cells) has, however, been assayed and found to be decreased²⁸.

Gangliosides (lipid NANA) are concentrated in the grey matter of the central nervous system and are associated with maintenance of electrical activity and ion transport²⁹. Subcellular studies show gangliosides in the microsomal fraction, presumably with fragments of dendrites and nerve endings^{29,30}. $\text{Na}^+ + \text{K}^+ - \text{ATPase}$ has a similar distribution²⁹⁻³² and is closely associated with the appearance of electrical phenomena in the cerebrum¹⁵.

Brain $\text{Na}^+ + \text{K}^+ - \text{ATPase}$ activity is depressed in hypothyroidism in rats and primates while a reduction in ganglioside content has only been observed in this study. The report of a normal content of ganglioside in the hypothyroid rat cerebrum is not explained considering that the same workers described reduced dendritic arborization³³ and evidence for a reduction of synaptic terminals^{1,34}.

Examination of hypothyroid rat cerebral cortex by interference microscopy and planimetry reveals significant reduction

in the dry mass and area of cortical neurones³⁴. This agrees with our findings of a reduced non-chloride space.

As anorexia is a common accompaniment of hypothyroidism and maternal malnutrition may cause changes in foetal brain growth, it can be asked whether the changes observed in this study are an effect of undernutrition. Female rhesus monkeys have been subjected to severe protein/calorie restriction during pregnancy. Preliminary results indicate that the cerebrum of their offspring is unaffected while minimal changes concerning myelination are seen in the cerebellum. Legrand's³⁵ histological studies of underfed and hypothyroid rat cerebellum suggest that abnormalities observed after neonatal hypothyroidism are specific for that condition.

From our results, one can conclude that overall RNA and protein synthesis is depressed in the foetal brain in the absence of thyroid hormone. Our data indicate that both neuronal and neuroglial cell populations are functionally affected in cerebrum and cerebellum. Further work with this primate model should afford a better understanding of the role of foetal thyroid hormone in the development of the brain.

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Identification of a Possible Subunit of Hexosaminidase A and B

THE two chief forms of N-acetyl- β -D-hexosaminidase of human tissues are distinguishable by their electrophoretic behaviour and heat stability and can be separated by ion exchange chromatography^{1,2}. These properties have been used in a differential assay of the forms for diagnostic purposes in cases of lysosomal storage disease. Tay-Sachs' disease is characterized by the lack of the thermolabile hexosaminidase A and by elevated amounts of the more stable hexosaminidase B³ and another form of the enzyme as yet incompletely characterized. In Sandhoff's disease⁴ which resembles Tay-Sachs' disease in the resultant accumulation of cerebral ganglioside GM₂, all forms of the enzyme are at a very low concentration. The A and B forms of the enzyme are closely related at a molecular level and antisera raised to either enzyme separated from human liver⁵ or from human placenta⁶ cross-react with the other enzymic form.

In our earlier reports we noted that hexosaminidase A could be converted to a form resembling but not necessarily identical to hexosaminidase B, by enzymic removal of sialic acid residues. This has led some authors^{7,8} to suggest that hexosaminidase B is a precursor of A and Tay-Sachs' disease represents a genetic lack of a specific sialo-transferase. No such transferase has yet been demonstrated, however, and the theory does not explain the spontaneous origin of new forms that arise during controlled denaturation⁹.

In order to explain these findings we proposed that the enzymes contain at least two different subunits¹⁰, one of which carries the active site for hydrolysis and is largely responsible for the observed antigenicity, being common to all forms of the enzyme, and the other of which is a "specifier" subunit characteristic of hexosaminidase A and conferring on the active site the specific ability to hydrolyse ganglioside GM₂. Tay-Sachs' disease would then represent a mutation of the specifier subunit, leaving hexosaminidase B unaffected, whereas Sandhoff's disease would result from loss of activity of the common enzymic subunit.

This biochemical model has been supported and reiterated recently by Beutler's report¹¹ of the presence of non-enzymic cross-reacting material, corresponding to forms A and B in liver from a patient with Sandhoff's disease. No cross-reacting material corresponding to the missing hexosaminidase A was found in Tay-Sachs' liver.

We have now detected and partially characterized a protein of low molecular weight, present in normal liver and in livers from patients with Sandhoff's disease and Tay-Sachs' disease, that has several of the expected characteristics of the proposed enzymic subunit.

Samples of Sandhoff, Tay-Sachs', and control livers were separated by gel permeation chromatography on columns of

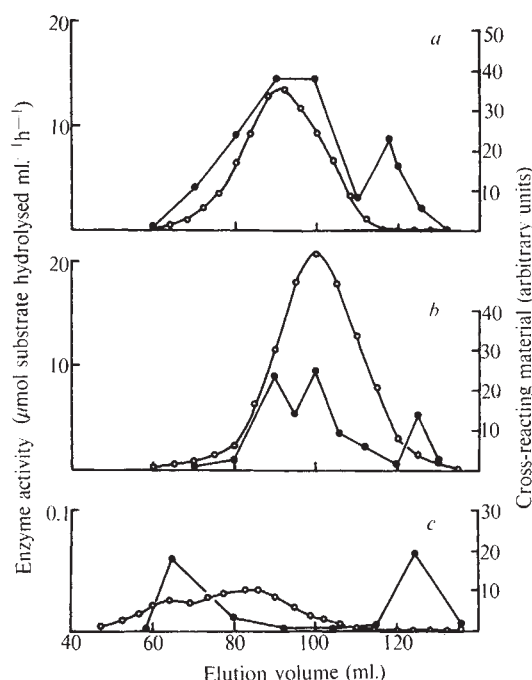


Fig. 1 Separation of hexosaminidases and cross-reacting material to antihexosaminidase A serum on 'Sephadex G-150'. a, Normal human liver; b, Tay Sachs' diseased liver; c, Sandhoff's diseased liver. ---, Hexosaminidase; —, cross-reacting material.

'Sephadex' G-150, and the eluate tested for hexosaminidase activity, using the synthetic substrate 4-methylumbelliferyl-N-acetyl- β -glucosaminide, and for cross-reactivity to antihexosaminidase A serum. Details of the preparation of the antigen for this antiserum have already been published, and it was shown to be devoid of hexosaminidase B and any other proteins of molecular weight lower than 10 (ref. 5).

It was found (Fig. 1) that the cross-reactivity occurred not only in those samples containing active hexosaminidase (molecular weight 130,000) but also in a component of low molecular weight, estimated to be 20,000–25,000 by comparison with marker proteins of known size. Sandhoff and Tay-Sachs' tissue also showed not only macromolecular cross-reacting material corresponding in size to the complete enzyme but considerable amounts of the low molecular weight material which was entirely devoid of hydrolytic activity towards the synthetic substrate. The macromolecular cross-reacting material of Sandhoff tissue cochromatographs with the low but measurable amount of activity present in these cases, whereas in Tay-Sachs' tissues it cannot be distinguished from the considerable amount of remaining hexosaminidase.

The material of low molecular weight, which is being further purified and characterized, has thus the following properties expected of the proposed enzymic subunit of hexosaminidase. Its molecular weight is a fraction of that of the complete enzyme, and it is serologically cross-reactive with antibodies to the complete enzyme. As the material cross-reacts with antiserum precipitating both hexosaminidase A and hexosaminidase B we assume that it has a structure closely related to common features of those two antigens. It can be predicted that the specific antihexosaminidase A serum described by Beutler could be used in a similar fashion to locate the specifier subunit characteristic of that enzyme if this theory is correct.

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Intrinsic Semiconduction, Electronic Conduction of Polymers and Blood Compatibility

CONSIDERABLE efforts are being made to understand better the interaction of blood with synthetic as well as natural surfaces and to develop materials that are compatible with blood¹⁻⁵. The compatibility of blood with surfaces has been associated chiefly with ionic charges based on the observations that the endothelial wall, the platelets and the plasma proteins carry net anionic charges in normal physiological conditions⁶. The possible role of electronic conduction and semiconduction in blood compatibility, by contrast with mere ionic interactions, has not, however, been demonstrated before. I describe here observations in support of such a role.

In 1946, Szent-Györgyi^{7,8} suggested that proteins may have an electronic structure similar to that of semiconductors. Evans and Gergely⁹ calculated that this is possible although they presented no conclusive evidence. In 1953, Eley *et al.*¹⁰ reported semiconductivity in albumin, fibrinogen, and edestin.

Synthetic polymers are typically insulators rather than conductors of electricity. Some polymers can be made "conductive" by the dispersion of finely powdered metal particles or carbons, or by coating them with metal oxides. Polyurethanes filled with carbon have been reported which showed higher thromboresistance than those without carbon¹¹. Although these and other polymers containing dis-

persed conducting particles are sometimes inaccurately referred to as "conducting" plastics, their conductivity is attributable to the presence of the dispersed extraneous particles within the polymer matrices rather than to the intrinsic structure of the macromolecules.

I have previously reported in detail the thermal conversion of a specially purified aromatic polyimide, poly[N,N'-(*p,p'*-oxydiphenylene)pyromellitimide], into semiconducting and conducting condensation products¹²⁻¹⁶. In addition to intrinsic electronic conduction and semiconduction, the brittle materials retain their original shapes after weight loss during pyrolysis, have smooth surfaces, contain no leachable impurities, and in the powdered state show unusual adsorptive properties in aqueous organic solutions¹⁷.

The thermal conversion process is accompanied by the appearance of an electron paramagnetic absorption (EPR) spectrum caused by the formation of free radicals¹³. The calculated free spin concentrations (g^{-1}) are greater than 10^{19} , and the relative EPR adsorption curves exhibit characteristic maxima as functions of time and temperature of the pyrolysis¹²⁻¹⁴. During the thermal conversion the resistivity of the system decreases (conductivity increases) and the density increases¹²⁻¹⁴. The thermal conversion process is accompanied by cleavage of the carbonyl groups of the polyimide and formation of condensed polynuclear aromatic and heterocyclic systems with enhanced π -orbital overlap and consequent increase in the mobility of electrons¹²⁻¹⁴.

In subsequent work, the Lee-White blood coagulation times, partial thromboplastin times (PTT), and platelet aggregations and activation were determined with human blood. The two former blood values were compared with those of siliconized glass tubes¹⁸. The partial thromboplastin times are indicative of the activation of blood coagulation factors. The data in Table 1 indicate that the semiconducting and conducting polymers have clotting times two to three times longer than that of siliconized glass, whereas the non-conducting original polyimide has a clotting time of approximately a third that of siliconized glass. The partial thromboplastin indices show similar trends. Using plasma rich in platelets¹⁹, the electroconducting and semiconducting polymers show very low or no platelet aggregation with no evidence of activation when compared with the original, insulating polyimide. No *in vivo* tests with animals have been carried out but they are planned in collaboration with others.

Although it is premature to generalize, if subsequent chemical, physical, and biological experiments further confirm the results, they will open up new horizons in the understanding of the interaction of blood with synthetic surfaces, leading to practical applications. They may also have implications as far as arteriosclerosis and the molecular properties of the vascular endothelium are concerned. It is possible that electroconduction and semiconduction are involved in the interaction of surfaces with plasma proteins, and activation of the Hageman factor and the platelets by an unknown mechanism.

Table 1 Physical and *in vitro* Biological Properties of Insulating, Semiconducting and Electroconducting Polypyromellitimides (PPMI)*

Sample	Density (g cm^{-3}) (refs. 12, 13)	Specific electroconductivity $\dagger$ (ohm cm^{-1}), 25° C (refs. 12, 13)	No. of spins g^{-1} (refs. 12, 13)	Lee-White coagulation index $\ddagger$	PTT index $\ddagger$	Platelet aggregation and activation
Unmodified PPMI	1.426	$< 10^{-12}$	—	0.3	0.5	Heavy and activated
Pyrolytic PPMI	1.33	0.1	$> 10^{19}$	2.1	1.3	Very light to none, not activated
Pyrolytic PPMI	1.48	11.1	$> 10^{19}$	2.6	1.3	Very light to none, not activated
Pyrolytic PPMI	1.65	20.0	$> 10^{19}$	2.5	1.4	Very light to none, not activated

* Poly[N,N'-(*p,p'*-oxydiphenylene)pyromellitimide].

$\dagger$ Specific electroconductivity = $1/\text{specific resistivity}$.

$\ddagger$ Expressed as ratio of clotting times for test specimens and those of siliconized glass (average of three).

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Intestinal Adenomatosis in the Pig: Occurrence of a Bacterium in Affected Cells

ENTEROPATHY of the weaned piglet, originally described by Biester and Schwarte¹, has been further reported by Rowland and Rowntree². Affected pigs show adenomatous proliferations of the intestinal mucosa, principally of the lower ileum and caecum. The affected tissue consists of glands lined by vigorously proliferating immature epithelial cells throughout the entire depth of the mucosa, with loss of villi and often the development of frankly polypoid masses. Goblet cells do not form. In the absence of secondary infection, most animals recover within about 6 weeks and the intestine is normal at slaughter. Histochemical studies have shown the adenomatous cells to be lacking the more common enzymic activities of normal mature villous epithelium.

Immunofluorescent studies, using a fluorescein isothiocyanate (FITC) conjugated serum prepared from an affected pig, appropriately absorbed with pig tissue, have demonstrated, in the cases so far examined, particulate fluorescence in the apical cytoplasm of affected cells throughout the depth of the mucosa. These animals originated from several farms.

Further proof of the specificity of this reaction is afforded by the removal of fluorescent activity from the serum after absorption with affected intestinal mucosa but not normal intestinal mucosa from the same animal.

Electron microscopic examination of conventionally prepared and cryostat material has consistently identified an irregularly curved bacterium within abnormal cells situated in the same areas in which fluorescence has been observed.

Attempts to identify the presence of these organisms by the usual bacterial stains, either in section or in smears of affected intestinal mucosa, have been unsuccessful. The ultrastructure of the intracellular bacterium is simple and we have been unable to observe any specialized cell structures, such as axial fibres, which have been present in intracellularly located spirochaetes in swine³.

Cultural examination of affected intestinal mucosa has resulted in the isolation of an organism with a similar morphology to that seen by the electron microscope in the adenomatous lesions. These bacteria have the characteristics of a vibrio, but initial studies suggest that the organisms differ biochemically from most vibrio species isolated from the pig by other workers⁴⁻⁷. The vibrios obtained from adenomatous lesions show a degree of antigenic homogeneity, although preliminary work indicates some antigenic differences between isolates. In contrast, none of these organisms is agglutinated significantly by a series of five antisera (unpublished results of G. H. K. L. and J. Hannah) prepared against whole cells of isolates considered to be *V. coli*, recovered from the large intestine of pigs. These latter have characteristics similar to those described by Lussier⁷. The vibrio occurring in the proliferative intestinal lesions has been demonstrated to be present in three cases, from which it has been isolated in numbers approximating to 15×10^6 per g weight wet tissue.

Hyperimmune serum against the vibrio isolates has demonstrated, using a "sandwich" technique, specific intracellular fluorescence at a similar site to that observed using conjugated porcine immune serum.

Within the cell, the organisms are not contained within vacuoles or other membrane-bound structures. This relationship seems unusual, although bacteria have recently been described free in the cytoplasm of affected hepatic cells of the Mongolian gerbil in Tyzzer's disease⁸.

One must be extremely cautious at this stage in drawing conclusions regarding any interaction between the intracellular bacteria and the proliferative intestinal changes observed, especially in view of the long and controversial history of the possible pathogenicity of various vibrio species in the pig. Even in the absence of specific cause and effect, however, the host-parasite relation in these circumstances may prove of considerable interest and possible significance.

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Pepsin Inhibitory Activity amongst Activation Peptides of Pepsinogen

THE concept of the pepsin inhibitor (PI) and a method for its isolation in crystalline form from the acid-activated pepsinogen (Pg) system, in which pepsin (P) and other peptides (pp)

are released ($\text{Pg} \rightarrow \text{P} + \text{PI} + \text{pp}$), were advanced by Herriott¹. PI was believed to interact with pepsin at pH 5 to 6, this inhibitory interaction being most readily observable in this pH range in the milk clotting system in which pepsin is believed to catalyse the reaction $\kappa\text{-casein} \rightarrow \text{para } \kappa\text{-casein} + \text{caseinomacropptide}$.

PI was found² to be a basic peptide of twenty-nine amino acid residues with leucine in the N-terminal position, but the amino acid composition originally reported does not now correspond to any single stretch of pepsinogen sequence as recently determined³.

We report here on the peptides separated by a chromatographic method after release from porcine pepsinogen activated at pH 2 for 1 min. One of the peptides (AP-V, Fig. 1), has pepsin inhibitory activity at pH 5.7 in the milk clotting system equivalent to the inhibitory activity of PI, but differs from PI in amino acid structure and in having fewer amino acid residues, although both have the same N-terminal amino acid, leucine.

Porcine pepsinogen, prepared according to Ryle⁴, was activated at pH 2; the released pepsin was inactivated and removed by filtration after precipitation by trichloroacetic acid and the residual activation mixture adjusted to pH 6 and chromatographed on CM-'Sephadex'. Salt (sodium acetate) gradient elution yielded fractions, each of which was examined for pepsin inhibitory activity in the milk clotting system¹, for ninhydrin reactivity⁵ and for absorbance at 280 nm. Fig. 1a, b shows the chromatographic separation which was obtained; chromatograms are separated for clarity.

The AP-V peptide fraction was isolated by freeze-drying after desalting on 'Sephadex' G-25 (using 2.5% acetic acid), and an amino acid analysis was carried out.

Inspection of Fig. 1a, b shows clearly that AP-V, having marked ninhydrin and milk clotting inhibitory activity, is of immediate significance and it was found to contain arginine (2), aspartic acid (1), glutamic acid (1), leucine (4), lysine (4), proline (1), serine (1), valine (3). Judging also by the absence of isoleucine, glycine, threonine, histidine, phenylalanine and tyrosine, it is equivalent to the first sixteen amino terminal residues of porcine pepsinogen reported³ to contain the highly basic -Arg-Lys-Lys- sequence.

Figure 1a shows that ninhydrin reactivity of the fractions is not necessarily accompanied by pepsin inhibitory activity and *vice versa*.

In spite of the sharing between AP-V and the crystalline PI of a common N-terminal leucine and equivalent pepsin inhibitory activity, the variety of amino acids and the number (twenty-nine) of amino acid residues reported by Van Vunakis and Herriott² together preclude common identity for these two inhibitors. Also, work³ on the amino acid sequence of pepsinogen reveals less correspondence between PI and pepsinogen than between AP-V and pepsinogen.

The distribution of pepsin inhibitory activity amongst activation peptides of pepsinogen is the subject of continuing investigation in this laboratory but it seems that very intense inhibitory activity, for example AP-XI (Fig. 1a, b), can be associated with what, on the basis of ninhydrin reactivity, might be concluded to be a minor peptide.

In another experiment using SE-'Sephadex' (after Rajagopalan *et al.*⁶) it has been possible to elute (0.1 M NH_4OH) basic peptides which are devoid of pepsin inhibitory activity. In similar work, but using 'Amberlite' IR-120, such peptides were found³ to contain at least one lysine or arginine residue, and even in one peptide the very basic -His-Lys-His- sequence. Neither of these two groups reported on pepsin

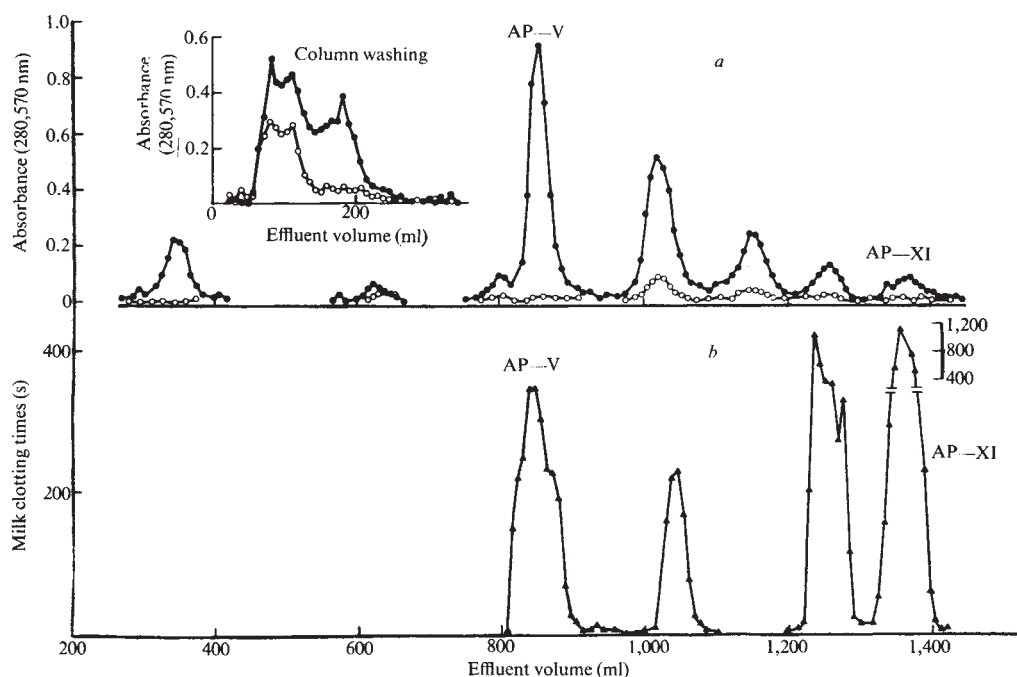


Fig. 1 Chromatography at 4° C of the activation peptides from 500 mg of chromatographically purified pepsinogen on a column (2.54 × 40 cm) of CM-'Sephadex' G-25 (Pharmacia Ltd, London; particle size 40–120 μm , capacity 4.5 ± 0.5 meq g^{-1}) equilibrated with 0.1 M sodium acetate buffer (pH 6.0). The peptides were produced by activation of pepsinogen (500 mg 35 ml^{-1} water; 5 N hydrochloric acid to pH 2.0, 1 min). Liberated pepsin was removed by filtration after denaturation (5 N sodium hydroxide to pH 11 to 12, 5 min) and precipitation (3 N trichloroacetic acid to pH 2.0, 20 min). The resulting solution (approximately 80 ml) so obtained was adjusted to pH 6.0 and applied to the column, which was then washed with 200–300 ml of the equilibrating buffer. Elution was carried out with an exponential acetate concentration gradient formed by passing 1 M sodium acetate buffer, pH 6.0, into a constant volume 1 l mixing vessel containing 1 l of 0.1 M sodium acetate, pH 6.0. Rate of flow was 30 ml h^{-1} and 2 ml fractions were collected. Pepsin inhibitory activity of 0.25 ml of the fractions was assayed by the milk clotting method of Herriott¹ modified by the use of a pepsin concentration of 0.2 mg ml^{-1} for convenience; milk clotting times (test-control) are a direct measure of pepsin inhibitory activity. Ninhydrin reactivity⁵ (0.5 ml aliquots) and absorbance at 280 nm of the same fractions were measured. ▲—▲, Milk clotting times (s); ●—●, ninhydrin reactivity (absorbance at 570 nm); ○—○, absorbance at 280 nm.

inhibitory activity for these basic peptides. Assuming that the non-inhibitory peptides separated in this laboratory are analogous, and owe their basic character, to the amino acids noted by Perlmann³, the absence of pepsin inhibitory activity which we find supports the contention that inhibitory interaction with pepsin at pH 5 to 6 requires in the peptide something more than the presence of basic amino acid residues, a fact that has been foreshadowed⁷ in pepsin inhibitory studies of lysine, which is inactive, and polylysines, in which inhibitory activity can, to some extent, be related to molecular weight⁸⁻¹⁰.

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Specific Neutralization of Human Hepatitis Type A in Marmoset Monkeys

THE transmission of human hepatitis type A (infectious hepatitis) to marmoset monkeys, previously reported from this laboratory¹⁻⁵, has been confirmed by several other laboratories⁶⁻⁹ but questioned by one^{10,11}. To strengthen the case for considering the agents causing experimental hepatitis in marmosets as viruses of human hepatitis type A, and not as latent "marmoset hepatitis viruses" as has been suggested by the dissenting laboratory^{10,11}, it was necessary both to show clearly the lack of induction of disease by normal human serum or plasma and to demonstrate specific neutralization by convalescent human serum of the hepatitis type A agents transmitted from man to marmosets. We describe such neutralization here.

After the original transmission of hepatitis type A to marmosets, several experiments to test the reliability of the system were performed with coded specimens. In these

Table 1 Inoculation of Coded Human Specimens into Marmosets

No. of experiments	Species of marmoset	Type of inoculum	Number of specimens	Marmosets hepatitis/inoculated
5	<i>S. fuscicollis</i> , <i>S. nigricollis</i>	N	8	0/52
		H	11	47/56
1	<i>S. (Oedipomidas)</i> <i>oedipus</i>	N	2	0/7
		H	2	5/9
Totals 6		N	10	0/59
		H	13	52/65

N, Normal human serum albumin or control human serum or plasma; H, acute phase hepatitis A serum or plasma.

Table 2 Cross-challenge of Marmosets with Various Hepatitis Isolates*

First inoculation type	Second inoculation type	Result
GB	GB	No disease
GB	Berlin	No disease
GB	MS-1	Hepatitis
Berlin	GB	No disease
WW-55	GB	No disease
MS-1	MS-1	No disease
MS-1	GB	Hepatitis

* For origin of hepatitis isolates see refs. 1, 3 and 7. All animals developed biochemical and morphological evidence of hepatitis after the first inoculation. They were reinoculated 2-12 months after their serum enzyme activities had returned to normal. Each animal was challenged with 10^3 - 10^4 infectious doses intravenously and was evaluated as previously described¹.

experiments human serum or plasma obtained during the acute phase of hepatitis regularly caused hepatitis in marmosets whereas control or pre-infection serum or plasma did not (Table 1). Other experiments established some of the physical and biochemical characteristics of one of the hepatitis virus isolates⁵. These characteristics (with the possible exception of heat stability) were similar to those established epidemiologically and by human volunteer studies of human hepatitis type A viruses¹². Similar results have been obtained recently with hepatitis type A viruses isolated in another laboratory¹³. Cross challenge of marmosets with various hepatitis isolates showed that there were at least two different antigenic types of hepatitis A viruses¹⁴ (Table 2). Yet these data were not sufficient to establish the identity of the agents isolated in marmosets as human hepatitis A viruses.

Attempts were therefore made to neutralize the disease in marmosets with convalescent human serum¹⁵. Acute phase serum or plasma from human volunteers inoculated with the MS-1 strain of human hepatitis A (ref. 16), or the infectious fractions of such serum or plasma prepared by density gradient centrifugation in caesium chloride², were mixed in a 1:1 ratio with pre-inoculation or convalescent serum from one of the volunteers. The mixtures were incubated for 16-18 h at 4° C, and were then inoculated into groups of marmosets (*Saguinus nigricollis*, *S. fuscicollis*);

Table 3 Neutralization of Hepatitis A in Marmosets

Inoculum	Marmosets hepatitis/inoculated	Average incubation period (days)
M _A + control	9/12	45
M _A + M _C	1/8	110
F + control	4/6	26
F + M _P	4/4	28
F + M _C	0/6	—
K _A + control	3/5	42
K _A + M _C	0/6	—

Control, human serum albumin; M_P, preinfection plasma from volunteer M; M_A, acute phase plasma from volunteer M; M_C, convalescent plasma (90 days postinoculation) from volunteer M; K_A, acute phase plasma from volunteer K; F, infectious plasma fraction. Three aliquots (5 ml. each) of a pool of acute phase plasma from three volunteers (M, K and R) who had been inoculated with the MS-1 strain of human hepatitis A were centrifuged at 30,000 r.p.m. for 3 h at 20° C in a SW-50 rotor of a model L Beckman ultracentrifuge. Each pellet was resuspended in 5.0 ml. of CsCl (1.1958 g ml.⁻¹) and was recentrifuged in the same rotor at 30,000 r.p.m. for 24 h at 20° C. Fractions were collected and fractions of the density range of 1.18-1.22 g ml.⁻¹ were pooled and bovine serum albumin was added to a final concentration of about 1%. The pool was then dialysed for 12 h against distilled water and for an additional 6 h against phosphate buffered saline at a pH of 7.2. The pool was mixed with the various materials as indicated in the Table and was incubated and inoculated as described in the text.

0.5 ml. of the mixture was administered intravenously to each animal. These experiments were performed under code, the code being kept by one of us (M. C.) until all results were recorded. The results are given in Table 3. Neither human serum albumin nor pre-inoculation serum inhibited the infectivity of the acute phase serum or its infectious fraction, whereas the convalescent serum neutralized the infectivity of these inocula almost completely.

Similar observations have been made simultaneously and independently in Dr Hilleman's laboratory with hepatitis isolates obtained from cases of epidemiologically proven hepatitis A occurring in Costa Rican children. These isolates were neutralized by convalescent but not pre-inoculation sera from the same group of volunteers (who had been inoculated with MS-1 hepatitis) used in our study, and they were also neutralized by the convalescent sera of contact cases from the Costa Rican study but not by pre-illness sera of the same individuals¹³.

The results of both investigations, and of previous studies, establish that human hepatitis type A viruses can be transmitted to some species of marmosets, inducing biochemically and morphologically typical hepatitis. This finding eliminates the need for human volunteers in most further studies of hepatitis A, and should lead to complete identification of and preventive measures against hepatitis type A viruses.

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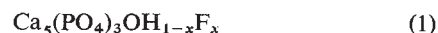
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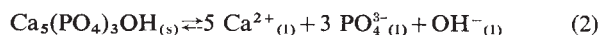
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Relation between Apatite Solubility and Anti-cariogenic Effect of Fluoride

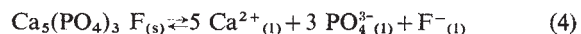
THE free enthalpy of formation of solid solutions of hydroxyapatite (OHA) and fluorapatite (FA) at 25° C



has been determined¹. I have used these thermodynamical data to calculate (Driessens, unpublished) the solubility of the solid solutions in aqueous solutions at certain pH values. For this purpose, the following relations were derived:



$$-\log K_{\text{OHA}}^1 = 5 p\text{Ca} + 3 p\text{PO}_4 + p\text{OH} = -\log K_{\text{OHA}} - \log a_{\text{OHA}} \quad (3)$$



$$-\log K_{\text{FA}}^1 = 5 p\text{Ca} + 3 p\text{PO}_4 + p\text{F} = -\log K_{\text{FA}} - \log a_{\text{FA}} \quad (5)$$

where subscript (s) means that the preceding molecular unit is part of the solid solution; subscript (l) means that the preceding ion is dissolved in the aqueous solution; K_{OHA}^1 and K_{FA}^1 are the apparent solubility products of the components OHA and FA over the solid solution respectively: they depend on x ; K_{OHA} and K_{FA} are the solubility products of pure OHA and pure FA respectively: they are independent of x ; a_{OHA} and a_{FA} are the thermodynamical activities of FA and OHA, which are sufficient to account for the dependence on x .

Data for the activities were derived by application of the appropriate graphical method to the literature data of the free enthalpy¹. Then the solubility (s gmol of apatite per litre) was calculated at pH 4, 6 and 8 as a function of x on the assumption

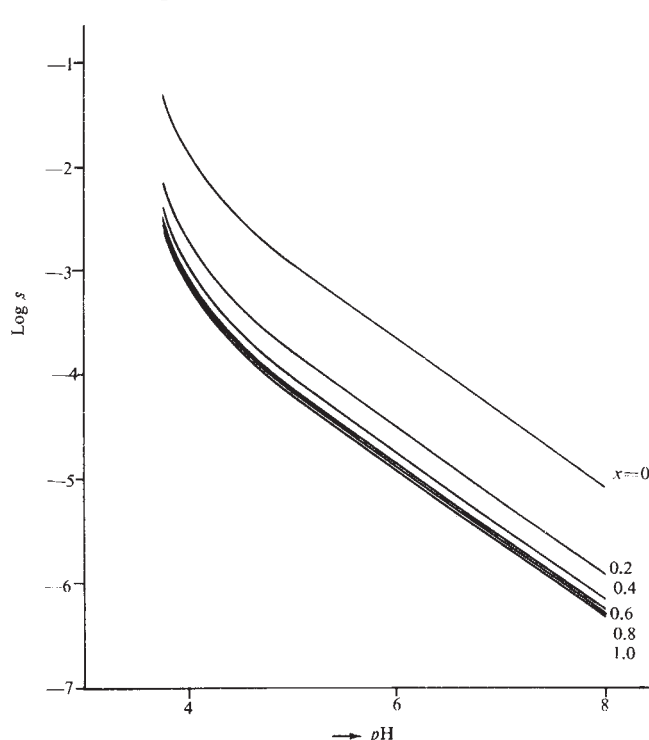


Fig. 1 Solubility s in gmol of apatite ($\text{Ca}_5(\text{PO}_4)_3\text{OH}_{1-x}\text{F}_x$) dissolved per litre at 25° C as a function of pH for a number of compositions x of the OHA-FA solid solutions at equilibrium.

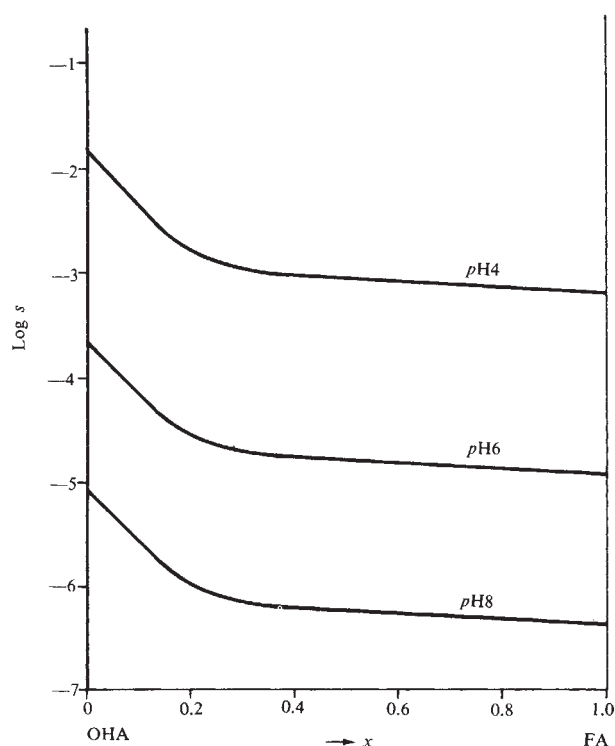


Fig. 2 Solubility s in gmol of apatite dissolved per litre at 25° C as a function of the composition x of the OHA-FA solid solution at pH 4, 6 and 8.

that the aqueous solution would not contain Ca^{2+} or phosphate ions other than from the original OHA-FA solid solution. If necessary, corrections were made for the occurrence of binuclear complexes, such as CaHPO_4^0 , $\text{CaH}_2\text{PO}_4^+$, in the aqueous solution. The results are given in Figs 1 and 2. Values for K_{OHA} and K_{FA} were taken from Moreno, Gregory and Brown² and McCann³. Values for the stability constants of binuclear complexes were taken from Gregory, Moreno and Brown⁴.

The calculated data show that the reduction of the solubility of hydroxyapatite at a certain pH in the range $4 \leq \text{pH} \leq 8$ by the incorporation of F^- ions is nearly independent of pH but depends strongly on x . The reduction of the solubility as compared to that of pure hydroxyapatite amounts to 44, 68, 80 and 87% at the following degrees of substitution of OH^- by F^- ions: $x=0.05, 0.10, 0.15$ and 0.20 respectively.

Although the apatite in tooth enamel is not a pure OHA-FA solid solution, it may be expected that the incorporation of small amounts of fluoride ions has at least the same effect on its solubility. In fact, detectable preventive action has been observed when drinking water contained 1 p.p.m. F^- . On the other hand, such a concentration and dosage lead to a value of about $x=0.05$ within a short period after eruption of the teeth⁶. Therefore, the reduction of the apatite solubility by F^- incorporation seems to be a sound basis to explain the anticariogenic effect of fluorides.

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Bacterial Production of Di-Methyl Nitrosamine in Salted Fish

THE presence of the potent experimental carcinogens, N-nitrosamines, in food and their possible formation during food processing have recently received much attention. N-nitrosamine formation from the interaction of nitrite with secondary amines in mammalian stomach^{1,2} and *in vitro* by the action of nitrate-reducing bacteria has been reported^{3,4}. This *in vitro* effect was confined to bacteria of the intestinal flora. Reports concerning N-nitrosation of secondary amines by bacteria isolated from food are scarce. Collins-Thompson *et al.*⁵ demonstrated the production of N-nitrosamines *in vitro* by bacteria isolated from meat products.

We have reported the presence of di-methyl nitrosamine (DMN) and di-ethyl nitrosamine (DEN) in appreciable quantities in Chinese salted marine fish obtained from markets in Hong Kong⁶. This was attributed to bacterial action on nitrate in the crude salt used to prepare salted fish. We have obtained viable culture on salt agar of nitrate-reducing *Staphylococcus aureus* and halobacteria.

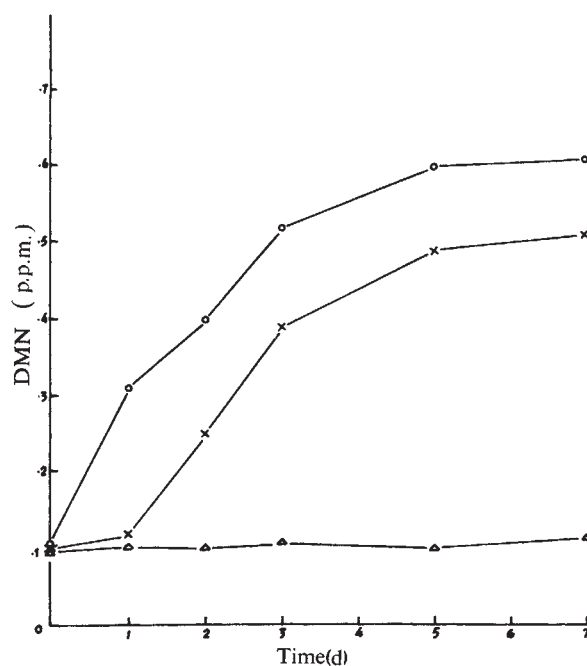


Fig. 1 Concentration of DMN in fish broth after incubation at 37° C. Δ , Portion A, sterilized; $\circ$, portion B, inoculated with 10^9 *Staphylococcus aureus* per lot; $\times$, portion C, untreated.

In this communication we report an increase in DMN in fish broth inoculated with the staphylococcus originally isolated from salted fish obtained from the market.

Chinese salted fish were cut into small pieces and homogenized with a Sorvall 'Omni' homogenizer. The homogenate was divided into three equal portions—A, B, and C. Each was subdivided into six equal lots in conical flasks. They were treated as follows. Portion A was sterilized by heating in a water bath at 70° C for 1 h daily. Portion B was sterilized by the above method on 0 day and 10^9 *Staphylococcus aureus* in 1 ml. were added to each lot. Portion C was untreated.

The nitrate content in the fish broth was 40 p.p.m. and nitrite content 5 p.p.m., estimated according to the method of Follet and Ratcliff⁷.

The material was incubated at 37° C. One lot from each

portion was taken out and analysed for DMN content on day 0, 1, 2, 3, 5 and 7. The method of extraction of DMN was described by Sen *et al.*⁸. For identification, a Varian aerograph model 2868 was used, and identification was done on two different columns: (a) 5% SE on 80–100 mesh high performance grade 'Chromosorb W (AW-DMCS)' $\frac{1}{8}$ inch $\times$ 6 foot stainless steel column. (b) 10% 'Carbowax 20 M' on 80–100 mesh high performance grade 'Chromosorb W (AW-DMCS)' $\frac{1}{8}$ inch $\times$ 10 foot stainless steel column.

The extract was further examined and the identity of DMN confirmed by the combined GC-MS technique. The concentration of DMN was calculated from the gas chromatogram peak area and the intensity of the molecular ion of the mass spectrum. The results are shown in Fig. 1. A shows no change in the DMN content of the fish broth; B and C show progressive increases, with the curve flattening towards the end of the 7-d period. The rise in the DMN content of portion C lags 1 d behind that of B, but thereafter C parallels B.

Our study shows conclusively the important role of the staphylococcus originally isolated from fish in the production of DMN from its precursors in the fish. The amount of DMN present would thus seem to be dependent on the storage condition, degree of contamination by nitrate-reducing bacteria, and the amount of precursors present. These features are in keeping with our observations (Fong and Chan, unpublished results) that different batches of fish from the market show a considerable variation in N-nitrosamine content. The study described here, however, does not resolve the question whether the part played by the bacteria is enzyme-dependent⁹ or non-enzymatic as reported by Collins-Thompson *et al.*⁵.

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Wear of English Monumental Brasses caused by Brass Rubbing

THERE are about 8,000 monumental brasses in the churches of Britain, representing only a small proportion of the total number of brasses set in place between about 1250 and 1650 (ref. 1). The preparation of rubbings of many of these brasses is extensively practised at present, particularly by visitors to England. Although brass rubbing (in the Netherlands) is documented as far back as 1656, the use of a wax crayon on paper has been popular in Britain only since the early nineteenth century². Thus, when viewed in historical perspective, the increasingly popular brass rubbing

activity must be considered as a comparatively new feature in the history of the monumental brasses. Our study was undertaken to determine whether repeated brass rubbing over periods of the order of a century will result in significant brass wear caused by the friction of the back of brass rubbing paper against the brass.

Neutron activation analysis is a sensitive means of measuring exceedingly small amounts of copper transferred from the surface of the brass to the back of the brass rubbing paper as a result of the microscopic motion of the paper against the brass during rubbing. The amount of transfer per unit area can be considered as a measure of the wear on the brass caused by making a rubbing.

Before beginning our experiments in Britain, we made several trial rubbing experiments at NBS on cleaned, freshly polished and freshly machined brass surfaces. On both kinds of brass surfaces measurable quantities of copper were transferred to the back of the paper. We report here



Fig. 1 Typical rubbing and associated control following sampling for neutron activation analysis. Two samples have been cut from the rubbed area and one from the control area. Head is of James de Hoveston, Blickling Church, Blickling, Norfolk (c. 1360).

a series of controlled experiments carried out on representative monumental brasses in Norfolk, England.

Multiple rubbings from ten brasses in four churches in Norfolk were made using white paper selected for its low content of Cu. We have shown in separate control neutron activation experiments that the black crayon used (Astral heelball) did not contribute significant amounts of Cu to the paper. Before making a rubbing, the brass was lightly

brushed with a clean brush and a piece of 8.5×11 inch paper, selected from the centre of the ream, was laid on the brass. One portion of the paper was constantly protected from the brass by interposing a second sheet of the same Cu-free paper; this protected section was used as an analytical control in each case. Fig. 1 shows a typical rubbing, and associated control, after removal of samples for neutron activation analysis. The brass was rubbed with heavy pressure until further rubbing caused no visible changes in the intensity of the rubbed regions. Each finished rubbing and its associated control were stored in separate sealed polyethylene bags without folding.

The sealed packets were opened at the National Bureau of Standards in a lamellar flow dust-free hood. Two or more square (25 cm^2) samples were cut from each sheet. Each sample was folded and rolled up, then hermetically sealed in a medical grade polyethylene tube. Seventy-two samples (rubblings plus controls) were prepared. Two standard solutions of copper were prepared by dissolving weighed quantities of high purity Cu metal in dilute HNO_3 , and these standard solutions as well as the samples were loaded into polyethylene "rabbits". Neutron activation inducing the reaction $^{63}\text{Cu}(n,\gamma)^{64}\text{Cu}$ was accomplished by irradiation for 60–120 s in RT-4 of the NBS 10 MW reactor (neutron flux $= 1.2 \times 10^{13} \text{ cm}^{-2} \text{ s}^{-1}$). After irradiation the samples were stored for $>16 \text{ h}$ to permit the decay of active short half-life radiation. Each sample was removed, folded flat, and sealed between two sheets of non-irradiated polyethylene, providing reproducible counting geometry. Known volumes of the standard irradiated copper solution were used to saturate folded filter paper which was counted in the same configuration.

The radiation counter was a Li-drifted Ge detector (2.4 keV resolution at 1.332 MeV). The amount of activity caused by decay of ^{64}Cu (half-life $= 12.8 \text{ h}$; detection of the 0.511 MeV γ -ray due to positron annihilation) was monitored for each sample during counting times of 2–5 min. The number of counts was corrected for a 0.511 MeV positron annihilation γ -ray arising from Na impurity in the sample. This correction was 2.5% of the number of counts in the 1.36 MeV Na peak, and ranged from 2–20% of the number of ^{64}Cu counts, depending on the copper content of the samples. The total number of ^{64}Cu counts in a 5 min interval was approximately 400 for a blank and up to 7,700 for a rubbed sample with a high content of Cu. All data were corrected for the decay of radioactivity in the time interval between irradiation and counting.

Table 1 shows the basic data obtained in this experiment. The fraction of the sample area rubbed in direct contact with the brass surface is determined by the extent of detail incised in the region of the brass selected for study; this parameter was measured after counting using the dark areas on the rubbing samples, and varied from 1.00 for smooth areas to 0.26 for highly decorative regions. The measured copper content has been tabulated on a normalized basis by dividing the total copper content found by the fractional area rubbed.

A total of twenty-seven control samples were analysed for Cu in this study, and these values were found to be remarkably uniform ($0.039 (\pm 0.007) \mu\text{g Cu cm}^{-2}$). In each of the forty-five rubbing samples studied the copper content was larger than that found in the associated blank.

After counting, the reverse side of each sample was carefully examined visually and, in certain cases, small flecks of gold or silver-coloured material were detected. Chemical tests have established that these metallic-coloured flecks are most likely due to tiny particles from crayons used in previous brass rubbing activities. Separate neutron activation measurements have shown that by contrast with black crayons, metallic-coloured crayons contain appreciable quantities of copper. Counting excised flecks from some

contaminated samples, we found that much of the total Cu content was contained in the flecks. (Samples in which such contamination is suspected are designated by an asterisk in Table 1.)

In Fig. 2 we show the frequency distribution of the data on Norfolk brasses. Data are separated into two sets, primary and secondary, based respectively on the visual absence or presence of metallic-coloured flecks. The primary set of rubbing samples has a median Cu content near $0.12 \mu\text{g Cu cm}^{-2}$ and a range from 0.018 – $0.39 \mu\text{g Cu cm}^{-2}$. The secondary set has a median Cu content near $0.25 \mu\text{g Cu cm}^{-2}$ and exhibits a broader distribution.

If we adopt the median value of about $0.12 \mu\text{g Cu cm}^{-2}$

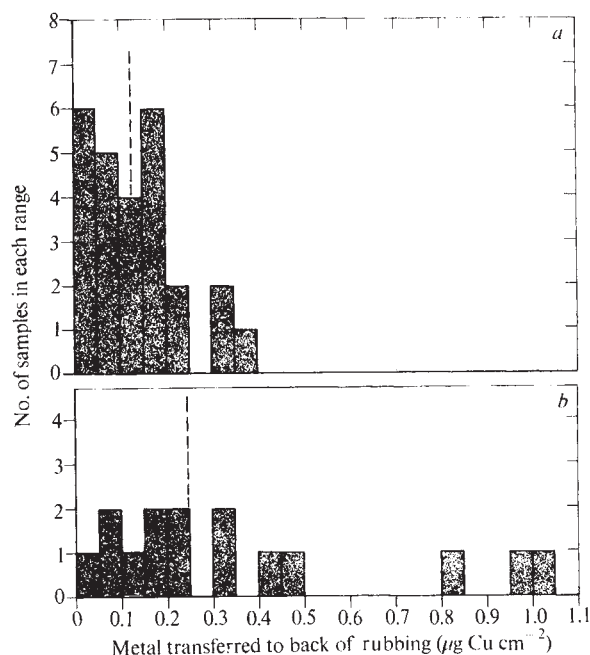


Fig. 2 Frequency distribution of copper transfer from English monumental brasses. ---, Median Cu transfer. *a*, Primary set of samples; *b*, secondary set, involving possible extraneous Cu contamination.

as typical of wear arising from brass rubbing, we can convert this number to a typical thickness of brass removed per rubbing. English monumental brasses of the period studied contain about 65–80% copper by weight³. Assuming a brass density of about 8.5 g cm^{-3} , we find that $0.12 \mu\text{g Cu cm}^{-2}$ corresponds to a thickness of about $1.8 \times 10^{-8} \text{ cm}$ of brass removed per rubbing, assuming uniform loss. If we assume that a popular brass will be rubbed three times per day on average, then in one century $\sim 10^5$ rubbings will be made, and the wear associated with this number of rubbings will amount to $\sim 1.8 \times 10^{-3} \text{ cm}$. This seems a trivial amount of wear by comparison with the usual depth of incision on many brasses ($\sim 0.1 \text{ cm}$), although the later (eighteenth century) copper plates sometimes have very shallow engraving⁴. Our primary set of data indicates a maximum Cu transfer about three times as great as the typical value chosen above. Even this maximum observed value of Cu transfer corresponds to a trivial amount of brass degradation over a century of brass rubbing at a rate of three rubbings per day.

There is a point of caution to be emphasized in the interpretation of these data, however. We have measured only one aspect of brass degradation caused by brass rubbing

Table 1 Measurement of Copper Transfer to Back of Brass Rubbing Paper for Representative Brasses in Norfolk

Date of brass manufacture	Brass identification, location and description	Fraction of sample area rubbed	Cu ($\mu\text{g cm}^{-2}$) (above control)
c. 1360	James de Hoveston, Blickling Church, Blickling		
	Head	0.80	0.035
	Head	0.90	0.018
1401	Sir Nicholas Dagworth, Blickling Church, Blickling		
	Stomach	0.96	0.49*
	Stomach	0.99	0.34*
	Stomach	0.97	0.197*
	Mail	0.47	0.127
	Mail	0.66	0.059
	Sword belt	0.74	0.087*
1416	Sir Simon Felbrigg, Church of St Margaret, Felbrigg		
	Face	0.95	0.154
	Banner	0.26	0.31
1432	Robert Baxter, Mayor, St Giles Church, Norwich		
	Hand	0.83	0.154
	Robe	0.80	0.20
	Robe, repeat	0.84	0.110
	Foot	0.82	0.317
	Foot	0.86	0.206
	Inscription	0.79	0.151
1432	Christiana Baxter, St Giles Church, Norwich		
	Head	0.91	0.176
	Hands	0.86	0.167
1458	Cecilie Boleyn, Blickling Church, Blickling		
	Skirt	0.80	0.97*
	Skirt	0.68	0.83*
	Skirt	0.65	0.322*
	Skirt	0.65	0.14
	Inscription	0.70	0.41*
	Inscription	0.70	0.39
1485	Isabel Boleyn, Blickling Church, Blickling		
	Skirt	0.75	0.224
	Skirt	0.62	0.156*
	Inscription	0.61	0.125
c. 1500	? Robert Gardiner, St Andrews Church, Norwich		
	Upper robe	0.84	0.043
	Upper robe	0.64	0.025
	Lower robe	0.97	0.041*
	Centre robe	0.81	1.05*
	Centre robe	0.81	0.054
	Hands	0.84	0.047
c. 1500	? Wife of Robert Gardiner, St Andrews Church, Norwich		
	Middle robe	0.71	0.073
	Lower robe	0.72	0.100
	Lower border	0.90	0.049
1612	Thomas Windham, Church of St Margaret, Felbrigg		
	Detail near hand	0.72	0.246*
	Detail near hand	0.73	0.228*
	Detail near hand	0.62	0.115*
	Hand	0.81	0.075
	Central region	0.72	0.072*
c. 1960	NBS sample, washed with ethanol		
	Smooth face	1.00	0.045
	Smooth face	1.00	0.019
	Rough face	1.00	0.066
	Rough face	1.00	0.051

* Possible contamination by metal flecks from brass rubbing crayons left from previous brass rubbings.

and the general access of brasses to the public. Other types of degradation must surely exist, such as wear by rugs which are trodden on and moved repeatedly, the effect of brass flexure (with possible associated work-hardening and eventual fracture (H. K. Cameron, personal communication)), and corrosion caused by skin acids. Our results must not therefore be interpreted as indicating that monumental brass degradation from all possible causes added together is necessarily trivial.

We thank Dr H. K. Cameron, Mr John Smith, Mr John Page-Phillips, Rev. J. R. Southern, Rev. and Mrs Hedleigh Davis and Mr Tom Gills for their help in our study.

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¹ Gittings, C., *Brasses and Brass Rubbing*, 5 (Blandford Press Ltd, London, 1970).

² *Macklin's Monumental Brasses* (rewritten by Page-Phillips, J.), 108 (George Allen and Unwin Ltd, London, 1969).

³ Cameron, H. K., *Monumental Brass Soc. Trans.*, 8, 109 (1946).

⁴ *Macklin's Monumental Brasses*, 99 (1969).

Impurities in Saccharin and Bladder Cancer

WE reported¹ that a combination of dietary saccharin with a single dose of methylnitrosourea (MNU) produced bladder tumours in five out of twelve female Wistar rats. No tumours were observed in control animals receiving either saccharin or MNU alone. Since submitting this report, concern has been expressed at the United States Food and Drugs Administration meeting, May 17, that impurities in saccharin, particularly *o*-toluene sulphonamide, could be responsible for the bladder tumours observed in saccharin feeding studies at the Wisconsin Alumni Research Foundation (WARF)^{2,3}.

Saccharin manufactured by the same process as that used in the WARF studies, namely by the Remson-Fahlberg process, in which *o*-toluene sulphonamide is the starting material, is known to contain this compound as an impurity. A very recent analysis of the saccharin used in our experiment showed it contained 810 p.p.m. of *o*-toluene sulphonamide. No evidence is at present available that this level of *o*-toluene sulphonamide is significant, but the possibility that it is this, or some other impurity, rather than saccharin *per se* which acts as the co-carcinogen in our experiments, will be further investigated. The saccharin used in our work is representative of that currently sold not only in the UK, but also in many other parts of the world³.

We thank Dr A. W. Noltes, Coca Cola Europe, London, for arranging the analysis done by the Battelle Institute, Columbus, Ohio, USA.

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¹ Hicks, R. M., Wakefield, J. St. J., and Chowaniec, J., *Nature*, 243, 347 (1973).

² *Wall Street J.*, May 21, 1973.

³ *Food Chem. News*, 45, May 21, 1973.

BOOK REVIEWS

Predicting the Weather

Weather Forecasting as a Problem in Physics. By Andrei S. Monin. Translated by Paul Superak. Pp. x+199. (MIT: Cambridge, Massachusetts and London, December 1972.) \$12.50.

MOST readers of *Nature* will be aware that weather forecasting has recently been undergoing very substantial changes in technique. The older empirical and partly subjective methods (not lacking physics but unable to exploit it numerically to any great extent) have become largely, though by no means wholly, replaceable by quantitative methods deriving from direct application of the basic laws of physics. The problem posed is to observe the 3-D atmosphere, or some part of it, in regard to weather-significant properties like pressure, temperature, humidity at a given initial instant and then to calculate its evolution by using dynamical and thermodynamical equations and boundary conditions (a model) thought to represent, more or less adequately for the particular kind of forecast required, the physics of the evolutionary process. (For experimental purposes initial data may also be invented.) Because of the non-linearity of the equations the numerical solutions are only to be obtained by finite-difference methods on high speed, large capacity computers, so that massive computational as well as physical problems are involved.

The intensive development of the new techniques has been an important concern of atmospheric dynamical research since the Second World War and has involved academic as much as or more than weather-service scientists. (This research is blessedly almost free from categorization as "pure" or "applied".) But the only textual literature in English specifically on the subject has been that provided back in 1961 by P. D. Thompson's *Numerical Weather Analysis and Prediction* (Macmillan) so that great interest should be aroused by the appearance now of a second volume and that from a Soviet scientist who is very distinguished for his research on turbulence theory but had not perhaps been thought of in the West as greatly concerned with large-scale atmospheric dynamics. Dr Monin shows here that he is as familiar with our literature in this field as with his own to which he has indeed contributed significantly.

Monin's book is in four sections. A short introduction deals with the history of the subject and with the scales and spectra of atmospheric processes. This is followed by a chapter on the theory of short-range weather prediction which in physical terms is to use an adiabatic model and in practical terms a model useful for two or three days since the run-down time of the atmosphere is a week or so. The use of quasi-geostrophic, quasi-solenoidal approximations which filter out the "non weather producing" motions from the system is fully discussed and advocated against the current tendency to use the more complete ("primitive") equations.

The author then passes to problems of long-range prediction, global climate and climatic change, for which non-adiabatic processes—radiation, friction and turbulent fluxes—are very much at the heart of the matter. These latter, strangely enough for Monin, are not given much attention except for radiative heating and cooling. There are discussions of a global system of observation such as will be used for the Global Atmospheric Research Programme, of predictability, and of possible mechanisms for climatic change. A final section deals with laboratory modelling using differentially heated rotating liquids, with the atmospheres of the planets out to Jupiter and with the utilization of forecasts.

This is a scholarly work in which few words are wasted. There is indeed such compression, in parts describing the construction of models, that some familiarity with the field by the reader is desirable but at least he does not have to face large amounts of intricate mathematics. The writing is engagingly fresh with the author ready to express controversial views both about the science and its uses which will raise some eyebrows. Yet it also has a somewhat pedagogic quality with here and there a lack of balance, as if the writer were passing on received knowledge rather than the results of work with which he has been much concerned. The practising forecaster might thus find the presentation occasionally irksome and he would mainly seek in vain for examples of the applications of the techniques described, except in long term, global models. But the scope of the treatment is admirable and will most certainly be enjoyed by all with some knowledge of the subject. The book

should also be an excellent means of informing physicists who would like to be made aware without needless complication of the way in which the subject is heading.

The translation is reasonably idiomatic but one remains pleasantly aware that the work was originally written in Russian.

P. A. SHEPPARD

Visual Physiology

Physiology of Photoreceptor Organs. Edited by M. G. F. Fuortes. Pp. x+765. (Springer: Berlin and New York, 1972.) 244 DM; \$77.40.

"VISUAL science has undergone remarkable development in the past two decades. A generation of competent, vigorous workers, skilled in the use of the latest biophysical and biochemical techniques, has built a structure of new understanding on older foundations that puts this branch of science in the very forefront of neurophysiology." Thus aptly, H. K. Hartline, who so very appropriately contributes the introduction as well as an article with F. Ratliff, summarizes the significance of this new volume of the *Handbook of Sensory Physiology*. What more can a reviewer reasonably say in a short space, to show that all the essays are welcome and many of them thoughtful summaries of a busy subject? Fuortes has picked his topics and contributors well. The literature up to about 1970 is discussed and, of course, one can hear the voices and read the reviewers commenting on how dated some of the articles are already. That may be so for the specialist reading the essay of his specialty. But I do not find this especially disturbing. The requirements of a handbook such as this are that the bibliographies shall be accurate and extensive and the articles readable. Thus it is possible to find out what is going on in specialities adjacent to one's own.

For example essays such as that by A. Kropf on the structure and reactions of visual pigments and W. R. Levick on the receptive fields of retinal ganglion cells are welcome not so much to the specialist but for their readability by those primarily interested in the structure of the compound eye of insects (O. Trujillo-Cenoz) or retinal metabolism (W. Sickel).

In all there are 18 chapters and this volume can fairly share the shelves with its great German progenitors. But whose shelves? It is understandable that publishers are not philanthropists and that book production of this standard is expensive. Yet it is hard to comprehend the economics of publishing such a generally useful book at over £30 a copy. If the answer be that libraries will buy it, the reply will soon be not all that many, at this price in the present waning economic circumstances of libraries. Perhaps *Nature's* readership could come up with some ideas for publishers for different costing, before more of us find the only way we can economically get a useful new book on our scholarly shelves is to review it briefly.

B. B. BOYCOTT

Neural Biochemistry

Neurotransmitters and Metabolic Regulation. Organized and edited by R. M. S. Smellie. Pp. ix+169. (The Biochemical Society: London, 1972.) £4; \$12.

THE organizers of the symposium upon which the present volume is based, and the editor, should be congratulated for having produced a well balanced and up to date collection of reviews on biochemical aspects of neurotransmission. The eight chapters cover all of the known neurotransmitter substances, and at the same time pinpoint many of the topics of greatest current research interest in the field as a whole. Thoenen gives an admirable review of the new area concerning the neurally mediated control of enzyme synthesis in adrenergic neurones. He discusses the effects of trans-synaptic influences and the protein nerve growth factor in controlling the activities of the biosynthetic enzymes tyrosine hydroxylase and dopamine- β -hydroxylase in adrenergic neurones. Watkins describes, mainly from a biochemical viewpoint, the metabolism of the amino-acid transmitters, glycine, gamma-aminobutyrate, aspartate and glutamate, and discusses the biochemical complexities resulting from the transmitter and metabolic functions of these substances, and how these functions may be related. McIlwain, and Greengard and McAfee, deal with the roles of adenine nucleotides, such as adenine, adenosine and cyclic AMP, in mediating the actions of neurotransmitters and in controlling neuronal excitability, a complex and fast-changing literature with a paucity of good reviews of this type. Whittaker and his colleagues, and Smith, discuss recent knowledge of the biochemical mechanisms involved in the uptake, storage and release of acetylcholine (in electric fish and in squid) and of noradrenaline in sympathetic nerves. For

these systems, and for other transmitters, remarkable similarities in the basic design of the biochemical mechanisms are emerging. In cholinergic and adrenergic nerves special uptake systems exist for choline and for noradrenaline respectively; the intraneuronal storage vesicles in both types of nerve contain not only high concentrations of the transmitter substances but also soluble proteins (chromogranins and vesiculine) so far with ill-defined but probable functions in transmitter storage. Banks and Mayor review an equally topical field, the axonal transport of macromolecules and cell organelles, illustrating by reference to their studies in adrenergic neurones. Carlsson and his colleagues review the regulation of the synthesis and degradation of 5-hydroxytryptamine by nerve activity in tryptaminergic neurones in mammalian central nervous system, and the various control mechanisms that may exist in such neurones.

Altogether the volume comprises a timely and useful collection of topical reviews of key areas, at a modest price. The volume is clearly illustrated and well produced, and should provide much valuable information to those involved in research or study in this rapidly growing field.

L. L. IVERSEN

Bacteriocins A-Z

The Bacteriocins. By P. Reeves. Pp. xi+242. (Chapman and Hall: London; Springer: Berlin and New York, March 1973.) £5.80.

BACTERIOCINS, the antibacterial agents produced by bacteria, have been of continuing interest to bacteriologists and biochemists since their discovery in 1925. Many early investigations concerned the identification of new types and their medical implications, such as the role of bacteriocins in recovery from infection and in control of normal flora. Currently, the mode of killing by colicins, the agents formed by *E. coli*, is of particular interest at the biochemical level, as is the phenomenon whereby a bacteriocinogenic bacterium is partly immune to its own bacteriocin. In many species, the genetic determinants of bacteriocin synthesis have turned out to be carried on autonomous extrachromosomal genetic elements—plasmids—many of which are related to other bacterial plasmids like the F factor or the drug-resistance factors of enterobacteria. Plasmid molecules have revealed new structures for replicating DNA and new forms of control of DNA replication.

This monograph has seven chapters dealing with bacteriocins in general and with colicins in particular; the

chemistry of bacteriocins; a shortish passage on the structure and behaviour of the genetic determinants; and much fuller sections on the adsorption of bacteriocins to bacteria and how they cause death. An appendix lists the known bacteriocins down to rarities like the "convexins" of *Eggerthella convexa*. The twenty-one-page bibliography includes titles as well as the last page number of each paper. Monographs can be all-embracing or written to a theme. This one is undoubtedly of the first sort, and will be very useful to everyone working in the field.

G. G. MEYNELL

Physics of the Sea

Marine Physics. By R. E. Craig. Pp. vii+83. (Academic: New York and London, February 1973.) £1.90.

THE difficulty in reviewing this little book is to know on what level to assess it. One of its aims, as given by the author, is to provide some background on physical aspects of the sea for engineers and biologists. He anticipates criticism of the unevenness of its treatment by stating that the different chapters do not depend greatly on each other: each is an essay on its own topic.

The term "density currents", in the first chapter, is taken to include large-scale geostrophic currents maintained by density differences in the ocean but the brief treatment, even when supplemented by later chapters, fails to indicate the importance of such flows. The most detailed chapter is that on wind currents in deep water, which gives a quantitative account of simple types of motion in the presence of Coriolis forces, leading on to atmospheric and oceanic flows involving viscous stresses. This is the classical Ekman spiral treatment with some original touches. It is taken for granted in this chapter that the reader is familiar with differential equations, but that on waves starts by assuming a cycloidal surface wave form in deep water with closed circular orbits and the phase velocity and attenuation with depth are derived on this basis. The equations of motion and continuity are not mentioned and there is no reference to the existence of a mass transport. The chapter on tides avoids almost entirely any quantitative treatment and gives a rather superficial descriptive account.

The last two chapters, on optics and acoustics, are elementary but soundly based and best meet the description of being brief, self-contained essays on their respective topics. The lack of consistency of treatment would make this book of limited value as a textbook, but taking it as a collection of essays on topics in marine physics the discriminating reader may find some items of interest.

K. F. BOWDEN